University of Alberta

Systematics and biology of the genus *Chrysomyxa* (Uredinales)

by

Patricia Ellen Crane ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Biological Sciences

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Systematics and biology of the genus* Chrysomyxa *(Uredinales)* submitted by *Patricia Ellen Crane* in partial fulfillment of the requirements for the degree of *Doctor of Philosophy*.

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Abstract

Rust fungi in the genus *Chrysomyxa* (Uredinales, Coleosporiaceae) occur in boreal forests of the Northern Hemisphere on conifers (mostly *Picea* spp.) and alternate to members of the Ericaceae *sensu lato*, especially *Rhododendron* (including *Ledum*). The 19 recognized species occurring in North America and Europe were studied by light and scanning electron microscopy, and, where possible, field observations and inoculation experiments. Detailed, fully illustrated descriptions are given, including taxonomic history, life cycle, and economic importance. In addition to host specificity, morphological characters useful in delineating species are size and surface morphology of aeciospores and urediniospores, including shape and size of warts and presence and nature of a longitudinal groove or cap, and morphology of the aecial peridium. North American rusts formerly considered varieties of the European *C. ledi* are recognized as distinct species: *C. nagodhii* on *L. groenlandicum* and *L. decumbens*, *C. neoglandulosi* on *L. glandulosum*, and *C. vaccinii* on *Vaccinium parvifolium*. A new small-spored species, *C. reticulata*, infecting *L. groenlandicum* and cultivated rhododendrons in North America is described. A new anamorphic species that lacks spermogonia and likely belongs to *Chrysomyxa* is described from coastal British Columbia as *Peridermium zilleri*. *Chrysomyxa ledicola* is shown to vary with location in urediniospore and aeciospore size, the presence of a narrow flat area on spores, and the presence of spermogonia.

Methods for DNA extraction and amplification of the ITS region of ribosomal DNA were investigated. Direct sequencing of ITS region amplification products was not

possible, suggesting the existence of variation among ITS repeat units within an individual.

The biology of selected species was elucidated experimentally. The life cycle of *C. woroninii* was confirmed by inoculation of basidiospores from *L. groenlandicum* onto spruce needles. Field observations and experiments with *Pyrola asarifolia* infected with *C. pirolata* showed that moisture is an important factor in the induction of telia on *Pyrola* leaves. The autoecious North American spruce rust, *C. weirii*, was investigated for morphology, spore dispersal and germination, and cytology. The monokaryotic teliospores are diaspores that germinate in free water to form a two-celled basidium and two tetranucleate basidiospores. Water dispersal of teliospores has not previously been reported in *Chrysomyxa*.

For Adrian, Stephanie, and Jonathan

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TABLE OF CONTENTS

Chapter **Page**

1. An overview of the genus *Chrysomyxa* 1

Hosts and life cycles 1

Taxonomic history and systematic position 2

Economic importance 4

Problems and objectives 5

Literature cited 7

Part I. Taxonomy and Systematics

2. Morphology, taxonomy, and nomenclature of *Chrysomyxa* species occurring in North America and Europe 11

Introduction 11

Materials and methods 13

Characters useful in taxonomy of *Chrysomyxa* 15

Taxonomic decisions and phylogenetic hypotheses 17

Keys to the identification of *Chrysomyxa* species occurring in North America and Europe 20

Species descriptions 25

C. abietis 25

C. arctostaphyli 27

C. cassandrae 31

C. chiogenis 36

C. empetri 39

C. ilicina 44

C. ledi 45

C. ledicola 49

C. monesis 58

C. nagodhii 60

C. neoglandulosi 65

C. piperiana 69

C. pirolata 71
C. reticulata 82
C. rhododendri 86
C. roanensis 91
C. vaccinii 94
C. weirii 95
C. woroninii 98
A new *Peridermium* on *Picea sitchensis*, *Peridermium zilleri* 101
Literature cited 153
3. The use of molecular characters in the systematics of *Chrysomyxa* 164
Introduction 164
Materials and methods 165
Results and discussion 169
Literature cited 173
Part II. Biological studies of selected species of *Chrysomyxa*
4. Clarification of the life cycle of *Chrysomyxa woroninii* on *Ledum* and *Picea* 176
Introduction 176
Materials and methods 178
Results 179
Discussion 183
Literature cited 189
5. Evidence for environmental determination of uredinia and telia production in *Chrysomyxa pirolata* (inland spruce cone rust) 192
Introduction 192
Materials and methods 193
Results 197
Discussion 203
Literature cited 211

6. Reproductive biology and evidence for water dispersal of teliospores in *Chrysomyxa weirii*, a microcyclic spruce needle rust 213
Introduction 213
Materials and methods 214
Results 216
Discussion 220
Literature cited 234
7. Conclusions and future research directions 237
Systematic considerations 237
Value of basic biological studies and field observations 238
Biogeography 239
Rusts as "plant taxonomists" 240
Future studies 241
Literature cited 243
APPENDIX: Glossary 245

LIST OF TABLES

Table **Page**

2.1. Comparison of the aecial stage of *C. nagodhii* and *C. cassandrae* 35

2.2. Comparison of *C. empetri* and *C. ledicola* on spruce 43

2.3. Comparison of *C. ledicola* from three regions of North America 53

2.4. Comparison of mean urediniospore size of *C. pirolata* on several hosts 76

3.1. Specimens used for DNA extraction and amplification 167

4.1. Details of inoculation experiments with *Chrysomyxa woroninii* 180

5.1. Summary of the artificial conditions used to test the effect of high humidity on the development of telia in systemically infected *Pyrola asarifolia* 195

5.2. Comparison of sorus development in covered and control *Pyrola asarifolia* plants infected with *Chrysomyxa pirolata* 201

LIST OF FIGURES

Figure **Page**

1.1. Life cycle of *Chrysomyxa ledicola*, a typical heteroecious macrocyclic species 6

2.1. Schematic tree showing hypotheses of the relationships of *Chrysomyxa* species in North America and Europe 105

2.2. *Chrysomyxa abietis* 107

2.3. *Chrysomyxa arctostaphyli* 109

2.4. *Chrysomyxa cassandrae* 111

2.5. *Chrysomyxa chiogenis* 113

2.6. *Chrysomyxa empetri* 115

2.7. *Chrysomyxa ilicina* 117

2.8. *Chrysomyxa ledi* 119

2.9. Comparison of mean spore size of urediniospores and aeciospores of *C. reticulata*, *C. chiogenis*, *C. ledi s.s.*, *C. cassandrae*, *C. neoglandulosi*, *C. nagodhii*, and *C. vaccinii* 120

2.10. *Chrysomyxa ledicola* on *Ledum groenlandicum* 122

2.11. *Chrysomyxa ledicola* on *Picea* spp. 124

2.12. *Chrysomyxa monesis* 126

2.13. *Chrysomyxa nagodhii* 128

2.14. *Chrysomyxa neoglandulosi* 130

2.15. *Chrysomyxa piperiana* on *Rhododendron macrophyllum* 132

2.16. *Chrysomyxa piperiana* on *Picea sitchensis* 134

2.17. *Chrysomyxa pirolata* on *Pyrola* spp. 136

2.18. *Chrysomyxa pirolata* on *Picea* spp. 138

2.19. *Chrysomyxa reticulata* 140

2.20. *Chrysomyxa rhododendri* 142

2.21. *Chrysomyxa roanensis* 144

2.22. *Chrysomyxa vaccinii* on *Vaccinium parvifolium* 146

2.23. *Chrysomyxa weirii* on *Picea glauca* 148

2.24. *Chrysomyxa woroninii* 150

2.25. *Peridermium zilleri* on *Picea sitchensis* 152

3.1. Portion of rDNA showing annealing sites of primers used 171

3.2. Comparison of ITS sequences of *C. empetri* and *C. arctostaphyli* 172

4.1. Witches' broom on *Ledum groenlandicum* caused by *C. woroninii* 187

4.2. Telia of *C. woroninii* on *Ledum* leaf 187

4.3. Intercellular hyphae of *C. woroninii* in stem of *Ledum* 187

4.4. Hyphae of *C. woroninii* below dormant bud of *Ledum* 187

4.5. Systemically infected spruce buds after inoculation with *C. woroninii* 187

5.1. Percentage of *Pyrola asarifolia* plants infected with *C. pirolata*, at wet and dry sites, that had greater than 50% of leaf surface covered with telia 206

5.2.-5.6. *Chrysomyxa pirolata* on *Pyrola asarifolia*. (5.2) Undifferentiated gelatinous sori. (5.3) Comparison of sorus development in adjacent plants, one left in field, the other with artificially induced telia. (5.4, 5.5, 5.6) Sori on same leaf before and after placement in a moist chamber 208

5.7. Uredinia of *C. pirolata* converting to telia 210

6.1. Sections of spruce needles infected with *Chrysomyxa weirii* 226

6.2.-6.5. Line drawings of spore morphology and germination of *C. weirii* on glass slides. (6.2) Teliospores. (6.3) Germination to produce two-celled basidium. (6.4) Basidiospore germination. (6.5) Other types of teliospore germination . 228

6.6. SEM micrographs of germinating teliospores and basidiospores of *C. weirii* on current-year spruce needles 230

6.7. Nuclear cycle of *C. weirii* shown by hematoxylin and DAPI staining 232

6.8. Schematic drawing of major nuclear events in the life cycle of *C. weirii* 233

LIST OF ABBREVIATIONS

ALTA	Vascular Plant Herbarium, Department of Biological Sciences, University of Alberta, Edmonton, AB
anam.	anamorph
CAFB	Vascular Plant Herbarium, Northern Forestry Centre, Edmonton, AB
CFB	Mycological Herbarium, Northern Forestry Centre, Edmonton,
comm.	communicated by
CTAB	cetyltrimethylammonium bromide
DAOM	National Mycological Herbarium, Agriculture and Agri-food Canada, Ottawa, ON
DAPI	4',6-diamidino-2-phenylindole
DAVFP	Mycological Herbarium, Pacific Forestry Centre, Victoria, BC
D.B.O.S.	D.B.O. Savile
DNA	deoxyribonucleic acid
FAA	formalin - acetic acid - alcohol
K	Herbarium, Royal Botanic Gardens, Kew, U.K.
ITS	internal transcribed spacer region of the nuclear ribosomal DNA
LM	light microscope (microscopy)
nom. nud.	*nomen nudum*, invalid name
NY	Herbarium, New York Botanical Garden, New York
PCR	polymerase chain reaction
P.E.C.	P.E. Crane
p.p.	*pro parte*, in part

PUR	Arthur Herbarium, Purdue University, Lafayette, Indiana
rDNA	nuclear ribosomal DNA
SEM	scanning electron microscope (microscopy)
s.l.	*sensu lato*, in the broad sense
s.s.	*sensu stricto*, in the strict sense
TE buffer	10 mM Tris-HCl / 0.1 mM EDTA, pH 8.0
TFM	Herbarium, Forestry and Forest Products Research Institute, Ibaraki, Japan
TSH	Herbarium, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan
WINF(M)	Part of CFB, formerly Mycological Herbarium, Canadian Forest Service, Winnipeg, MN
Y.H.	Y. Hiratsuka
W.G.Z.	W.G. Ziller

LIST OF SYMBOLS

!	After a specimen number, indicates specimen seen.
=	A facultative synonym; i.e. based on a different nomenclatural type, and judged by the taxonomist to be of the same organism.
≡	An obligate synonym, i.e. one that is based on the same nomenclatural type.

Chapter 1. An overview of the genus *Chrysomyxa*

Rust fungi (Basidiomycetes, Uredinales) are obligate parasites of ferns, conifers, and angiosperms. They have narrow host ranges, generally being confined to a single species or a few closely related plant species. Because of this, they are thought to have evolved closely with their host plants, and host relationships are very important in species concepts of rust fungi and in considering their evolutionary origin. Rust life cycles are complex, consisting of as many as five or six morphologically and functionally different spore states, either on one type of host plant (autoecious) or distributed between two unrelated host plants (heteroecious). Their fruiting structures consist of simple spore-producing structures called sori. Therefore, there are often few morphological characters to distinguish among species.

HOSTS AND LIFE CYCLES IN THE GENUS *Chrysomyxa*

In heteroecious species of *Chrysomyxa*, the telia are found almost exclusively on plants in the family Ericaceae *s.l.*, including subfamilies Rhododendroideae, Empetraceae, Pyroloideae, and Vaccinioideae (Anderberg 1993; Judd and Kron 1993; Cullings 1994; Kron 1996, 1997). The aecial stage of heteroecious species, and the telia of autoecious species, occur on needles, buds, and cones mainly of spruce (*Picea* spp.). Two species have also been described on hemlock (*Tsuga* spp.) and one on *Keteleeria*, a subtropical conifer with 11 species in China. All of these conifer hosts belong to subfamily Abietoideae of family Pinaceae (LePage 1993), demonstrating the narrow host ranges of these fungi. Although the two host families, Ericaceae and Pinaceae, are not phylogenetically closely related, they are closely associated ecologically in boreal forests of the Northern Hemisphere. Many of the ericaceous hosts have evergreen leaves that allow mycelium of the rust to overwinter in living tissue, which is essential to the survival of these obligate parasites in northern or subalpine areas. Many species can survive in the diploid stage in the angiosperm host in the absence of the conifer host; however, the conifer host is required for sexual recombination to occur.

About 30 species of rust fungi are currently considered part of the genus *Chrysomyxa*. Several other rusts likely belong in the genus, but are known only in the aecial stage; therefore they have been described in the anamorph genus *Peridermium* (Jen 1957; Bakshi and Singh 1967; Wang et al. 1980; Li 1986; Chen 1992). Durrieu (1984) refers to two additional taxa from southern Asia that have not yet been described. Most species of *Chrysomyxa* are heteroecious and macrocyclic (having five spore states) (Fig. 1.1). Six species are autoecious, with a single spore state, the telium, on the needles of various conifers. These have probably evolved from heteroecious macrocyclic ancestors (Jackson 1931; Cummins and Hiratsuka 1983).

The genus *Chrysomyxa* is mostly confined to the Northern Hemisphere. About one-third of the species have a circumboreal distribution and are well adapted to the short growing season of the subarctic; others are endemic to subalpine or temperate regions of the Himalayas or boreal forest regions of North America, Europe, and Asia. At least 14 species are found only in Asia, whereas 10 occur exclusively in North America.

About one-half of *Chrysomyxa* species infect *Rhododendron* spp. (including *Ledum*). The greatest diversity of rhododendrons (about two-thirds of known species) occurs in southeast Asia, western China, Tibet, and the Himalayas (Leppik 1974; Durrieu 1984; Irving and Hebda 1993). The rugged topography and complex habitat types consisting of tropical, subtropical, temperate, and alpine-arctic species distributed vertically over short distances, favors a large diversity of plants and their parasites (Zhuang 1993). In contrast, the small number of native rhododendrons and *Chrysomyxa* species (all circumboreal) in Europe may indicate that these plants, along with their rusts, migrated to Europe from southeast Asia after the Pleistocene glaciation (Leppik 1974).

TAXONOMIC HISTORY AND SYSTEMATIC POSITION

The genus *Chrysomyxa* was established by Unger (1840) based on the type species *C. abietis* (Wallr.) Unger, an autoecious rust that occurs on spruce needles in Europe and Asia. De Bary (1879) later included heteroecious species with their telia on *Ledum* and *Rhododendron*. Others created special genera or subgenera to reflect

differences in life cycle. Schröter (1879) reserved *Chrysomyxa* for *C. abietis*, but placed two heteroecious European species, *C. ledi* and *C. rhododendri*, in *Melampsoropsis*, a subgenus of *Coleosporium*. Schröter (1889) later described these variants as *Euchrysomyxa* (heteroecious macrocyclic), *Hemichrysomyxa* (aecia unknown), or *Leptochrysomyxa* (microcyclic). Arthur (1907) elevated *Melampsoropsis* to a genus, including only the macrocyclic species in both Europe and North America. He subsequently abandoned *Melampsoropsis* (Arthur 1925), recognizing that short- and long-cycled rusts are often closely related, based on similar morphology and occurrence on the same or closely related host plants.

Certain species have at times been placed in other genera, based on a single character. For example, Dietel (1890) created the genus *Barclayella* to accommodate an Asian autoecious needle rust that has telia similar to *Chrysomyxa*, because he did not observe basidiospores on small hyphal extensions known as sterigmata. This species was later placed in *Chrysomyxa* (Jaczewski 1926). Chen (1984) described a new genus, *Stilbechrysomyxa*, to accommodate three Asian species with stalked telia. This name has not generally been accepted (Singh et al. 1987; Hiratsuka et al. 1992; Farr et al. 1996).

The suprageneric classification of rusts is controversial, with the genera divided among 2 to 16 families, depending on the researcher. Classification of the rust fungi is based mainly on morphological characteristics of teliospores (probasidia), the stage considered to be the teleomorph (Greuter et al. 1994). Rusts have traditionally been divided into two families, Melampsoraceae and Pucciniaceae, based on whether the teliospores are sessile or stalked (Dietel 1928; Arthur 1934). The genus *Chrysomyxa* is generally included in Melampsoraceae *s.l.* Various authors have subdivided this family into several subfamilies or tribes, or additional families have been described to accommodate some of the genera. Gäumann (1959) placed *Chrysomyxa* in its own family, Chrysomyxeae, whereas Azbukina (1974) placed it in subfamily Chrysomyxoideae, tribe Chrysomyxeae of Melampsoraceae. Cummins and Hiratsuka (1983, 1984) emphasized the morphology and position of the spermogonia in addition to telial morphology in classifying the rusts. They assigned *Chrysomyxa*, together with

Coleosporium, to the family Coleosporiaceae. In addition to the thin-walled unicellular teliospores, this family is characterized by subepidermal, determinate spermogonia, well-developed aecial peridium (anamorph genus *Peridermium*), aeciospores and urediniospores that are catenulate and verrucose, and hosts in the Pinaceae. In addition, the telia of *Chrysomyxa* are pulvinate or tonguelike in shape, and have a gelatinous or waxy appearance. They consist of chains of teliospores that are produced from the base of the sorus by thallic sporogenesis (Berndt 1999). The teliospores form in spring and germinate without a period of dormancy to form elongated, septate basidia.

Few detailed studies of the phylogenetic placement of *Chrysomyxa* have been done. A cladistic analysis of rust genera from several families, based on morphology and ontogeny, placed *Chrysomyxa* on a clade with *Coleosporium*, *Cronartium*, and *Endocronartium* (Hart 1988). Similar morphology of the haustoria supports a position near other tree rust genera, including *Hyalopsora*, *Milesina*, *Uredinopsis* (fir rusts), *Cronartium*, *Coleosporium* (pine rusts), and *Pucciniastrum* (spruce rust) (Berndt and Oberwinkler 1997). Comparison of sequences of the 5.8S region of ribosomal DNA of a small number of rust genera placed *Chrysomyxa* closer to *Cronartium*, *Pucciniastrum*, and *Melampsoridium* than to *Puccinia* or *Melampsora* (Kurkela et al. 1999).

ECONOMIC IMPORTANCE

The diseases caused by *Chrysomyxa* species can be economically important on both the broadleaved and conifer hosts. Damage such as defoliation, brooming, seed destruction, and reduced tree height and diameter are particularly severe in situations where the plants or the fungi are introduced into areas where they are non-native. For example, *Picea engelmannii*, a native North American spruce species has recently been introduced to Japan, where it has been severely damaged by endemic *C. abietis* (Takahashi and Saho 1985). Large seed losses occur in spruce seed orchards in the interior of British Columbia when they are located in areas where the broadleaved hosts of *C. pirolata* are common and provide a source of cone-infecting basidiospores

(Sutherland 1990, 1991). *Chrysomyxa* species that infect rhododendron hosts cause significant leaf disease on cultivated susceptible varieties (Gould 1966; Bennell 1985).

PROBLEMS AND OBJECTIVES

A comprehensive monograph for the genus *Chrysomyxa* is lacking. Savile (1950, 1955) described the North American species, but no revised account based on modern methods exists. The early descriptions by Barclay (1886*a*, *b*, 1890, 1891) are still among the most complete for species in the Himalayas. Although the life history is well-known for some species of *Chrysomyxa*, basic information on life cycles is lacking for others. Species on different continents are often similar, but they have not been adequately compared, and taxonomic affinities are unclear. Therefore a revision of the entire genus is needed, based on light and scanning electron microscopy, field observations, artificial inoculation, and DNA comparison. This basic information is essential to an understanding of the phylogeny of these fungi, their coevolution with their host plants, their relationship with other organisms in boreal ecosystems, and the identification and control of diseases. The objectives of this study were (1) to provide updated, detailed, well-illustrated descriptions of all species occurring in North America and Europe; (2) to discuss species relationships according to morphological criteria and host relationships; (3) to provide new information on basic biology of selected species; and (4) to investigate the use of DNA amplification and sequencing as tools for systematic studies of the genus.

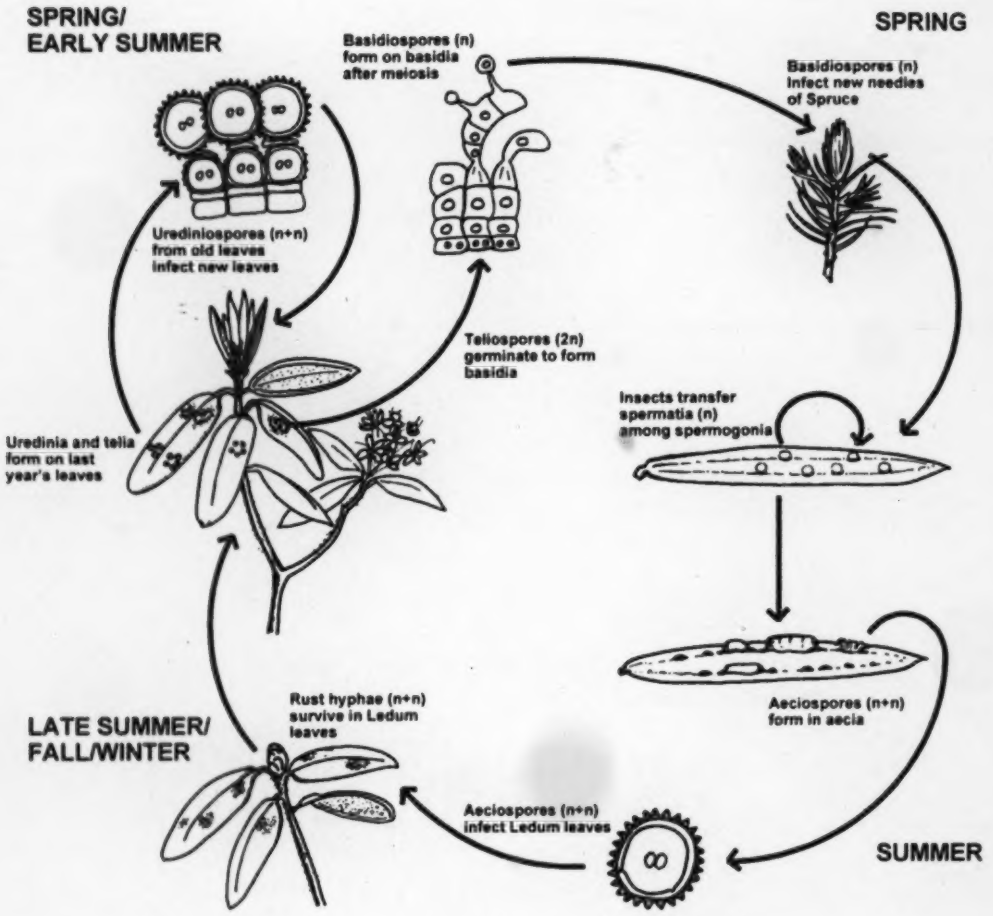

Fig. 1.1. The life cycle of *Chrysomyxa ledicola*, a typical heteroecious macrocyclic species.

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PART I. TAXONOMY AND SYSTEMATICS

Chapter 2. Morphology, taxonomy, and nomenclature of *Chrysomyxa* species occurring in North America and Europe

INTRODUCTION

Rust fungi (Uredinales) in the genus *Chrysomyxa* cause needle and cone diseases on conifers, mostly *Picea*. Two species have also been described on hemlock (*Tsuga*) and one on *Keteleeria*, a subtropical conifer with 11 species in China. All conifer hosts belong to subfamily Abietoideae of family Pinaceae (LePage 1993). Of the approximately 30 species in the genus, most are heteroecious, alternating to plants in the Ericaceae *s.l.* (including Rhododendroideae, Empetraceae, Vaccinioideae, and Pyroloideae). Many of these plants have evergreen leaves, in which the rusts overwinter. Some species have a circumboreal distribution, whereas others are endemic to subalpine or temperate regions of the Himalayas or to boreal forest regions of North America or Europe wherever their hosts occur. Depending on the species, location, and duration of infection, these rusts can cause severe defoliation, brooming, seed destruction, or reduced tree height and diameter, and may contribute to tree death. These fungi can be particularly damaging when spruce species are introduced into non-indigenous parts of the world (Takahashi and Saho 1985) or under intensive cultivation of host plants (Sutherland 1990, 1991; Singh and Carew 1990). Several *Chrysomyxa* species produce their uredinia and/or telia on cultivated rhododendrons and cause economically important diseases on those hosts (Gould et al. 1955; Gould 1966, 1967; Bennell 1985).

The genus *Chrysomyxa* was established in 1840 by Unger based on the type species *C. abietis* Unger, an autoecious rust that occurs on spruce needles in Europe and Asia, and produces only the telial stage. The telial sori are gelatinous or waxy, pulvinate or tonguelike structures that are erumpent through the host epidermis. Teliospores are one-celled, smooth-walled, and borne in chains. They germinate without dormancy to produce external basidia. Uredinia of most or all species are surrounded by an inconspicuous peridium consisting of one or more layers of thin-walled

pseudoparenchymatous cells (Berndt 1999). In *Chrysomyxa*, the urediniospores and aeciospores are both produced by basipetal succession to form chains of spores alternating with intercalary cells. With few exceptions, aeciospores and urediniospores in a given species have similar ornamentation, typically annulate warts, and this character can be used to infer relationship where the connection between spore states has not been experimentally proven. The aecia are peridermioid. Spermogonia are subepidermal and usually determinate and flask-shaped.

Identification of *Chrysomyxa* to the species level is often difficult, for several reasons. The telia provide few characters for distinguishing between species. Different heteroecious species may have similar urediniospore and aeciospore size, and they may occur on the same spruce species as well as the same telial hosts. Confusion also occurs because of the superficial resemblance of needle-infecting *Pucciniastrum* spp. to *Chrysomyxa* spp. However, several characters can be used to distinguish the aecia and spermogonia of the two genera. In *Pucciniastrum* needle rusts the spermogonia are pale, almost colorless, and subcuticular (seen in needle cross sections) (Hiratsuka and Cummins 1963), whereas in *Chrysomyxa* spp., the spermogonia tend to become a darkly pigmented brown or reddish brown as they age, and they arise subepidermally. *Pucciniastrum* needle infections appear earlier in the spring (June in western Canada) and aeciospores are pale yellow, whereas chrysomyxa rusts produce aecia later (July to August or even later in some species) and the aeciospores are salmon-colored to deep reddish orange, depending on the species.

Savile (1950, 1955) described the North American species of *Chrysomyxa*, but no well-illustrated, up-to-date account exists. Although the biology of certain species occurring in Europe is fairly well known, their relationship to species occurring elsewhere has not been adequately elucidated. A major objective of this study is to provide detailed, illustrated descriptions of the *Chrysomyxa* species that cause needle and cone rusts on *Picea* spp. and leaf or shoot diseases on broadleaved hosts, mainly in Ericaceae, in North America and Europe. On the basis of host and morphological differences, I consider the members of the *C. ledi* complex (Savile 1950, 1955) to be

separate species: *C. ledi* and *C. rhododendri* (de Bary 1879), and *C. cassandrae* (Tranzschel 1893) as originally defined; *C. ledi* var. *vaccinii* Savile as *C. vaccinii* comb. nov.; and the North American hypophyllous *Ledum* rusts as *C. neoglandulosi* sp. nov. (on *L. glandulosum*) and *C. nagodhii* sp. nov. (on *L. groenlandicum* and *L. decumbens*). Additional new species are *C. reticulata* sp. nov., previously unrecognized on *Ledum* spp. and cultivated rhododendrons in North America, and a new *Peridermium*, *P. zilleri* anam. nov., on *Picea sitchensis*. The latter most likely belongs to *Chrysomyxa*. An informal phylogenetic hypothesis (Fig. 2.1), based on morphology and host specificity, is given for the 19 species of *Chrysomyxa* described.

MATERIALS AND METHODS

Fresh material of most species was examined. Field observations were made of the natural development of all available spore states of *C. arctostaphyli*, *C. cassandrae*, *C. empetri*, *C. ledi*, *C. ledicola*, *C. nagodhii*, *C. neoglandulosi*, *C. pirolata*, *C. reticulata*, *C. weirii*, and *C. woroninii*. When fresh telia were available, spruce needles were artificially inoculated in a greenhouse or outdoors in Edmonton to obtain spermogonia and aecia connected with a particular ericaceous host. Natural infection by needle rusts at this location has not been observed. Disease-free spruce (*P. glauca* (Moench) Voss, *P. mariana* (Mill.) B.S.P., *P. pungens* Engelm., and/or *P. engelmannii* Parry ex Engelm.) grown from seed or transplanted from field locations were inoculated. Host leaves bearing telia were stored in a refrigerator at 4°C on moist filter paper in petri dishes. For inoculation, these leaves were placed, telia side down, directly onto needles of newly opened spruce buds, or several leaves or shoots were suspended above the tree on a wire cage so that basidiospores would be shed onto young needles. Trees were misted with distilled water, covered with a plastic bag for 48–72 h, then uncovered. They were observed for several weeks for disease development. For each experiment, several additional, uninoculated, trees were kept in the same location to serve as controls. In some cases, young spruce that were growing within 1 m of infected ericaceous hosts in the field were potted and kept in a greenhouse for observation of development of natural

infections. When authentic material on spruce was not available locally, material inoculated by earlier workers was obtained from various herbaria.

Herbarium specimens were examined from the following institutions: Northern Forestry Centre, Edmonton, Alberta (CFB, WINF(M), CAFB), and Pacific Forestry Centre, Victoria, B.C. (DAVFP), Canadian Forest Service; the Arthur Herbarium, Purdue University, Lafayette, Indiana (PUR); U.S. National Fungus Collection, Beltsville, Maryland (BPI); National Mycological Herbarium, Eastern Cereal and Oilseed Centre, Agriculture and Agri-food Canada, Ottawa (DAOM); the Royal Botanic Gardens, Kew, U.K. (K), the Vascular Plant Herbarium, Department of Biological Sciences, University of Alberta, Edmonton, AB (ALTA), the Finnish Forestry Research Institute, Rovaniemi, Finland; Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan (TSH). Under the species descriptions, only specimens for which slide mounts were made are listed; in most cases many more were examined for gross morphology with a dissecting microscope.

For light microscopy (LM), spores and hand sections of infected plant material were mounted in lactophenol or lactophenol – cotton blue. Spore measurements (15 – 20 spores per sample) were made at 400× using brightfield or phase-contrast microscopy; spermatia, wart height, and wall thickness were measured under oil at 1000×. When fresh material was available, basidiospores were collected by suspending leaves with telia above a microscope slide in a moist chamber. The basidiospores were measured without the apiculus. Measurements that were beyond the typical range are given in parentheses in the species descriptions. Spore color was determined for fresh material by comparison with Kornerup and Wanscher (1978) and recorded in the description as a color name and number.

For scanning electron microscopy, aeciospores or urediniospores were dusted onto aluminum stubs coated using adhesive tabs (Electron Microscopy Sciences), coated with gold, and examined with an Hitachi S-510 scanning electron microscope (SEM) operated at 15 or 25 kV. SEM images were captured using Quarz PCI software, version 4.0. Light microscope images were made by digitizing black and white photographic negatives

made from Kodak TMAX 100 or 400 film, or by digitizing 35-mm color slides and converting them to black and white. Adobe Photoshop 5.0 was used to adjust image contrast and to compose all plates.

CHARACTERS USEFUL IN TAXONOMY OF *Chrysomyxa*

Host specificity—As for most rust fungi, species of *Chrysomyxa* are usually adapted to specific hosts, and host identification is important in rust diagnosis on a broadleaved plant. For hosts in *Rhododendron* and *Ledum*, however, microscopic examination is imperative because several species of *Chrysomyxa* occur on species of both genera. Most species of *Chrysomyxa* in Europe and North America infect several species of spruce; therefore microscopic characters are almost always needed for identification of aecia.

Habit—The habit of a *Chrysomyxa* species on its telial host often reflects the habit on its aecial host, e.g. whether it is systemic or localized (Savile 1950). Awareness of this character may aid in elucidation of life cycles.

Aeciospores and urediniospores—Spore ornamentation is considered taxonomically useful at both the genus and species level in rust fungi (e.g. Hiratsuka and Kaneko 1975; Sato and Sato 1982; Lee and Kakishima 1999), and this was confirmed for the genus *Chrysomyxa*. Combinations of characters were emphasized in defining the species. The following characters were found to be fairly stable within most species: spore surface morphology, including the presence and characteristics of a groove, smooth area, or cap on the long axis; size and shape of warts (cylindrical, tapering, elongated, joined laterally); the nature of annuli on warts, and characteristics of leglike or weblike appendages at the base of, and often connecting the warts. Several of these characters, mainly elucidated by SEM, but sometimes visible by LM, have seldom been included in species descriptions for *Chrysomyxa* species. Additional characters that are useful, but may be quite variable under certain circumstances, are spore size and shape, wall thickness, and wart height. The distance between warts, a character used by Savile (1950, 1955), is very difficult to measure accurately by LM, and can vary greatly in a given

spore, especially within or adjacent to the above-mentioned grooves, or when warts are joined laterally. The position and number of germ pores, considered useful taxonomic characters at the species level in many rust fungi (Kaneko and Hiratsuka 1982), are not easily observed in *Chrysomyxa*. Even with special staining techniques (Holm 1965; Jennings et al. 1989), they were seen in only a few species.

Sorus morphology and aecial peridium—For several species, gross morphology of the aecia may be a useful character, including size and whether sori are confluent or not. The ornamentation of inner and outer peridial surfaces in addition to that of the aeciospores is useful in distinguishing among species. The importance of morphology of the aecial peridia was earlier recognized by de Bary (1879) in distinguishing between *C. ledi* and *C. rhododendri* in Europe. The surface morphology of the aecial peridium undoubtedly relates to the type of dehiscence, e.g. whether or not this structure is evanescent or persistent, and the dispersal mechanism of the aeciospores. Little is known about this mechanism for needle rust fungi.

Teliospores and basidiospores—Although teliospore size may be characteristic for each species, it is difficult to measure accurately because spores lengthen as they mature, and once they germinate to produce basidia and basidiospores, they collapse. In several species in the genus, teliospores are borne on sterile basal cells. This is an important diagnostic and taxonomic character. Lateral separation of the teliospores in a species may also be significant and has been observed in at least three species of *Chrysomyxa*: *C. weirii* and *C. roanensis* (this study), and *C. deformans* (Dietel 1890). Size and shape of basidiospores is another fairly consistent character within a species, but reports of size are affected by whether the apiculus was included.

Spermogonia—In *Chrysomyxa*, the spermogonia form along the stomatal lines of spruce needles. In rust fungi, the position of the spermogonia in relation to the host epidermis is diagnostic at the genus level (Hiratsuka 1963, 1980). In *Chrysomyxa*, these tiny structures are always subepidermal; they are usually determinate and and more or less globose or triangular in cross section, i.e. with a concave or flat hymenium (group I, type 1 or 2, Hiratsuka and Cummins 1963). Exceptions are the systemic cone rusts *C.*

pirolata and *C. monesis*, in which spermogonia are subepidermal, flat and possibly indeterminate (group IV, type 8, Hiratsuka and Cummins 1963). The width and height of the spermogonia, although somewhat variable, in conjunction with characters of the aecia and aeciospores may aid in a species diagnosis.

TAXONOMIC DECISIONS AND PHYLOGENETIC HYPOTHESES

Scanning electron microscopy has illuminated the morphological differences among the species formerly considered part of the *C. ledi* complex (Savile 1950, 1955). The name *C. ledi* was originally used for a European rust occurring on *Ledum palustre* L. and *Picea abies* (L.) Karst. De Bary (1879) documented its distinctiveness from *C. rhododendri*, also occurring on *P. abies*, but alternating to wild rhododendrons. Later the name *C. ledi* was applied to rust fungi in North America with similar life cycles (Arthur 1912; Fraser 1911, 1912) but occurring on different *Ledum* and *Picea* species. Savile (1950, 1955) noted subtle differences in urediniospore morphology among the rusts infecting *Ledum* spp. and other ericaceous plants, but contended that the aeciospores on spruce were indistinguishable and that they did not warrant species status. He therefore created six varieties of *C. ledi* de Bary to accommodate the rusts with similar spore size on *Rhododendron* spp., *Ledum* spp., *Chamaedaphne calyculata* (L.) Moench., and *Vaccinium parvifolium* Smith. Savile's variety names have been only partly accepted because telia were not described for all varieties. Variety names are often used for the uredinial states for several of these rusts, but the aecial states on spruce are called simply "*C. ledi* ". This has led to confusion in the identity of spruce needle diseases; it is hoped that the identification of the hypophyllous rusts of *Ledum* and *Rhododendron* will be facilitated by recognition that different species exist on different hosts and continents. Such recognition is increasingly important because of the transport of both spruce and rhododendrons around the world to locations where they are non-native.

The discovery of a previously unrecognized hypophyllous *Ledum* rust, *C. reticulata*, in North America may be horticulturally important. Evidence is presented that the rusts that appeared on cultivated rhododendrons on the west coast of North America

during the 1940's and 1950's (Gould et al. 1955; Savile 1973) transferred from native *Ledum* spp. rather than being imported from Europe on nursery stock. Urediniospores of *C. reticulata* on both host genera are smaller than other hypophyllous rusts on *Ledum* or *Rhododendron*, and they have a characteristic reticulate surface ornamentation over part of the spores. *Chrysomyxa reticulata*, as well as *C. nagodhii*, may have since spread elsewhere, such as Britain, because of the importation of nursery stock from the western United States (Bennell 1985). The ability of the same rusts to infect both host genera supports the recent morphological and molecular evidence that *Ledum* should be included in *Rhododendron* (Kron and Judd 1990; Harmaja 1991).

SEM also clearly showed the morphological differences between the aecial states of *C. ledicola* and *C. empetri*, species formerly thought to be almost indistinguishable on spruce (Savile 1950). In *C. ledicola*, however, the geographical variability in the following features is recognized for the first time: shape and size of urediniospores and aeciospores, wart size, presence or absence of a longitudinal indentation in urediniospores and aeciospores, presence or absence of spermogonia, and life cycle, i.e. whether secondary uredinia form during the summer. More work is needed to determine whether these variants represent discrete or intergrading units. Similarly, in *C. chiogenis* the differences in morphology between eastern and western North American samples warrant more study. This investigation confirms that *C. chiogenis* is probably closely related to *C. vaccinii*, as first suggested by Ziller (1974). For this reason, and others, the placement of *C. vaccinii* as a variety of *C. ledi* (Savile 1955) is rejected. Determination of the complete life cycle of *C. vaccinii* might clarify its relationship to *C. chiogenis* and to the anamorphic species *Peridermium zilleri*, which, like *C. chiogenis*, lacks spermogonia.

During this study, many specimens of *C. pirolata* were examined on different telial hosts in *Pyrola* and *Moneses*. In spite of some variability in surface ornamentation of the urediniospores, these differences could not be correlated with specific hosts, nor could significant differences in spore size be found. Nevertheless, field observations of the rust infecting only one host species when others were present in the same area (by myself and by W.G. Ziller) may suggest that pathotypes do exist in *C. pirolata*.

Variability in the occurrence of secondary uredinia during the growing season and in the size and crowding of these sori appear to be affected both by the host species and by the collection location. For this reason, and because no corresponding differences in spore morphology could be found among these variants, it seems best to consider all of them as *C. pirolata*. Therefore the separation of the rust on *P. secunda* as a separate species, *C. ramischiae*, based on its behavior on the host (repeating uredinia) (Lagerheim 1909), is not supported.

Similar peridial and aeciospore morphology, along with closely related hosts, supports hypotheses of phylogenetic relationship (Fig. 2.1). In three species, *C. ledicola*, *C. ledi*, and *C. neoglandulosi*, the peridial cells have a deeply concave outer surface and coarsely striate lateral walls, features that are easily observed by light microscopy. This morphology, along with their telial hosts in the genus *Ledum*, suggests a close relationship among these taxa (Fig. 2.1). Although the aecial peridium in *Chrysomyxa* species is typically white, brownish apical cells were observed in *C. roanensis* and *C. piperiana*. Both occur on native North American rhododendrons and their aeciospores are also similar in shape and surface morphology. Interestingly, each is confined to an isolated host and locality on opposite sides of the continent. They likely arose from a common ancestor and have begun to diverge on their respective rhododendron hosts after geographical isolation.

Although examination of Asian species was not a primary focus of this study, specimens of some of the studied species that also occur on that continent were compared for morphological consistency with those from North America and Europe. *Chrysomyxa cassandrae* and *C. empetri* from northern Japan and the Russian Far East were consistent with those from elsewhere; *C. rhododendri* from China was also similar to European and North American samples. However, several interesting anomalies were noted in rusts from Japan associated with *Ledum* and *Rhododendron* hosts. Samples from Japan identified as *C. ledi* and *C. rhododendri* were different in urediniospore size and ornamentation from European and North American specimens. On the other hand, *C. ledicola* from Japan was consistent with samples from the west coast of North America.

Thorough study of these rusts might lead to a new understanding of the biogeography and evolution of fungi in the genus *Chrysomyxa*.

This study describes and illustrates about two-thirds of the known species in the genus *Chrysomyxa*. Similar comparisons are needed with and among the species endemic to Asia. Southeast Asia and adjacent areas is considered to be the site of origin of both the conifer and rhododendron hosts of these parasites, and is therefore the likely origin of this rust genus (Leppik 1974; Wang 1984; Zhuang 1993). Study of the rust species from Asia may clarify the significance of the two telial types occurring in the genus, i.e. those with stalked and those with unstalked telia. Chen (1984) placed *C. himalensis* Barkl., *C. succinea* Tranz., and *C. stilbae* Wang et al. in the genus *Stilbechrysomyxa* on the basis of the stalked telia, but did not include *C. tsugae-yunnanensis* Teng., which also has this character. Another species with stalked telia, *C. qilianensis* Wang et al. (1987), has since been described. Two other species, *C. abietis* and *C. pirolata*, do not have conspicuous telial stalks, but the teliospores arise from elongated sterile basal cells similar to the above species. Because *C. abietis* has this character and is the type species of the whole genus *Chrysomyxa*, all species in the genus should be considered before a decision is made on the validity of *Stilbechrysomyxa*. In addition, the systematic position of the Asian microcyclic species *Ceropsora piceae* (formerly *Chrysomyxa piceae* Barclay), also occurring on spruce and having sterile basal cells in the telium (Bakshi and Singh 1960, 1967), requires reexamination.

KEYS TO THE IDENTIFICATION OF *Chrysomyxa* SPECIES OCCURRING IN NORTH AMERICA AND EUROPE

In this work, 19 species of *Chrysomyxa*, including 3 new species and one new combination, are illustrated and described. In addition, a new anamorphic species, *Peridermium zilleri*, is described from the west coast of North America. Keys to the aecial states on spruce and the uredinial/telial states are as follows.

Key to the aecial states on spruce

1. Infection systemic (causing disease or sporulating on entire buds, shoots, or cones) . 2
1. Infection localized (sporulating on only some needles, leaves or cone scales) 5
2. Aecia and spermogonia on systemically infected cones 3
2. Aecia and spermogonia on systemically infected buds or shoots 4
3. Aeciospore warts pulvinate, polygonal or elongated, crowded ..... *C. pirolata*
3. Aeciospore warts fluted, narrow or joined laterally, not crowded ..... *C. monesis*
4. On newly opened vegetative or reproductive buds ..... *C. woroninii*
4. On needles of perennial witches' brooms ..... *C. arctostaphyli*
5. Spermogonia lacking 6
5. Spermogonia present 8
6. Aeciospores (24)29–50 × 18–36 μm; peridial cells with coarsely striate side walls, fine shallow warts on inside surface ..... *C. ledicola* (west coast)
6. Aeciospores 20–44 × 10–19 μm; peridial cells without obviously striate side walls, densely warted on inside surface 7
7. Aeciospores fusiform with caps on one or both ends, part of a broad longitudinal smooth area ..... *Peridermium zilleri*
7. Aeciospores ellipsoidal or ovoid, smooth at ends ..... *C. chiogenis*
8. Aeciospores mostly <20 μm long, with a smooth longitudinal cap over part of spore .. .. *C. reticulata*
8. Aeciospores mostly >20 μm long 9
9. Aeciospores mostly rounded rather than elongated, no groove or smooth area 10
9. Aeciospores variously shaped, with a groove, cap, or smooth area over part of spore .11
10. Aeciospores >25 μm long, warts very coarse with obvious annuli ..... *C. ledicola*
10. Aeciospores mostly <25 μm long, warts very narrow and tapering, almost echinulate ..... *C. neoglandulosi*
11. Aeciospores usually <35 μm long 12
11. Aeciospores usually >35 μm long 15

12. Aeciospores without a groove, but having an obvious cap at one or both ends 13

12. Aeciospores with a narrow longitudinal groove or an indistinct smooth area 14

13. Aeciospores with a distinct broad longitudinal cap with a ragged edge ..... *C. cassandrae*

13. Aeciospores with a distinct longitudinal cap with a smooth edge ..... *C. nagodhii*

14. Aeciospores 18–30 × 16–22 μm, with an indistinct longitudinal smooth stripe, warts elsewhere cylindrical ..... *C. rhododendri*

14. Aeciospores 20–38 × 15–28 μm, with a narrow, well-defined longitudinal groove; warts narrow and tapering ..... *C. ledi*

15. Aeciospores fusiform, ellipsoidal or ovoid, 27–50 μm long 16

15. Aeciospores very long and narrow, fusiform, falcate, or lanceolate, 54–114 μm long ..... *C. piperiana*

16. Aeciospores with very tiny, shallow warts and an indistinct longitudinal smoother area over part of spore ..... *C. empetri*

16. Aeciospores with coarse cylindrical warts and a rough, broad distinct cap covering one-third to one-half of spore ..... *C. roanensis*

Key to the uredinial and telial states

1. Telia on needles of *Picea* 2

1. Telia and/or uredinia on a broadleaved host 3

2. Mature teliospores separate readily, not borne on sterile basal cells ..... *C. weirii*

2. Mature teliospores remain in sorus, borne on branched sterile basal cells ... *C. abietis*

3. Telia and/or uredinia on *Rhododendron* or *Ledum* 4

3. Telia and/or uredinia on a host other than *Rhododendron* or *Ledum* 12

4. Infection systemic in witches' brooms, telia on new shoots ..... *C. woroninii*

4. Infection localized 5

5. Telia and/or uredinia epiphyllous ..... *C. ledicola*

5. Telia and/or uredinia mostly hypophyllous, occasionally caulicolous 6

6. Urediniospores appear almost smooth on surface ..... *C. nagodhii*

6. Urediniospores with separate warts on surface 7

7. Urediniospores mostly globose or subglobose, with very fine, even warts on surface, no groove, cap, or smooth area ..... *C. neoglandulosi*

7. Urediniospores with a longitudinal groove, cap, or smooth area 8

8. Urediniospores subglobose, ellipsoidal, or ovoid, 15–36 × 12–26 μm 9

8. Urediniospores much longer than wide, 26–78 × 15–30 μm 11

9. Urediniospores with a narrow longitudinal groove, elsewhere warts crowded and tapering ..... *C. ledi*

9. Urediniospores with a poorly defined smoother area 10

10. Urediniospores 15–24(–26) × 12–21 μm, with a reticulate area over part of spore ..... *C. reticulata*

10. Urediniospores 18–32(–36) × 14–22 μm, often with a cap at one or both ends, part of a poorly defined longitudinal smoother area ..... *C. rhododendri*

11. Urediniospores very long and narrow, ellipsoidal to fusiform or cylindrical, with a very narrow longitudinal groove or cap ..... *C. piperiana*

11. Urediniospores ellipsoidal or lenticular; 1/3–1/2 of spore covered with a smoother area with crowded shallow bumps ..... *C. roanensis*

12. Produces systemic infection in *Moneses* or *Pyrola* 13

12. Produces localized infection; urediniospores and/or teliospores hypophyllous in a host other than *Moneses* or *Pyrola* 14

13. Urediniospore warts pulvinate, polygonal or elongated, crowded ..... *C. pirolata*

13. Urediniospore warts fluted, narrow or joined laterally, not crowded ..... *C. monesis*

14. Uredinia lacking, telia on *Arctostaphylos* ..... *C. arctostaphyli*

14. Uredinia and/or telia on a plant other than *Arctostaphylos* 15

15. On *Ilex* ..... *C. ilicina*

15. On *Gaultheria*, *Vaccinium*, *Chamaedaphne*, or *Empetrum* 16

16. Urediniospores 23–46 × 19–36 μm, on *Empetrum* ..... *C. empetri*

16. Urediniospores 15–33 × 11–24 μm 17

17. On *Chamaedaphne*, urediniospores with a broad cap at one or both ends .....
..... *C. cassandrae*

17. On *Vaccinium* or *Gaultheria* 18

18. Urediniospores tend to be elongated, ellipsoidal, ovoid, or oblong; on *Vaccinium* ...
..... *C. vaccinii*

18. Urediniospores tend to be globose, occasionally elongated; on *Gaultheria* .....
..... *C. chiogenis*

SPECIES DESCRIPTIONS

C. abietis (Wallr.) Unger, Beitr. Vergleichenden Pathol., p. 24. 1840. **Fig. 2.2, A–C**

=*Blennoria abietis* Wallr., Allg. Forst. Jagdztg. No. 17: 65–68. 1834.

=*Uredo epidermoidalis* Hartig, Verh. Harzer Forstvereins, p. 61. 1864. [*fide* Saccardo 7: 762. 1888.]

Type: On *Picea abies* (L.) Karst. near Grätz [Graz] in Styria, Austria, F. Unger.

Hosts and distribution: Strictly a Eurasian species found in Fennoscandia, Russia, Germany, Austria, Hungary, Switzerland, France, Belgium, Bulgaria, Poland (Sydow 1915; Jørstad 1936; Hylander et al. 1953), United Kingdom (Collins 1976; Mordue and Gibson 1978), China (Cao et al. 1996), and Japan (Hiratsuka et al. 1992). It has been reported on spruce species endemic to these areas, as well as introduced species, such as cultivated American spruces: *P. abies*, *P. engelmannii*, *P. glauca*, *P. pungens*, *P. rubens*, *P. sitchensis*, *P. glehnii* (Fr. Schmidt) Masters, *P. koyamai* Shirasawa, *P. omorica*, *P. shirasawae* Hayashi, *P. jezoensis* Carr. var. *hondoensis* (Mayr) Rehder, and *P. wilsonii* Mast.

Description: On *Picea*. *Spermogonia*, *aecia*, and *uredinia* unknown. *Telia* on distinct colored bands on needles of the previous year; amphigenous, but mostly on abaxial surface, pulvinate, arising along stomatal lines, orange-red, gelatinous, 0.5–10 mm wide; in cross section, constricted at base. *Teliospores* oblong to obovate with truncate base, 16–44 × 6–14 μm, produced in chains on sterile branching basal cells; wall colorless, thin. *Basidia* four-celled. *Basidiospores* 6–12 × 5–10 μm.

Notes: *Chrysomyxa abietis* is the type species of the genus. The waxy or gelatinous telia provide few characters that permit a precise placement of this species phylogenetically. Most *Chrysomyxa* species are heteroecious and macrocyclic, but because of the similarity of the telia, they have been placed in the same genus. However, *C. abietis* has a unique telial character not shared by most of the heteroecious taxa—elongated, sterile, dikaryotic basal cells on which the teliospores are produced (Fig. 2.2, A, B). Other species with this character are endemic to Asia, and include *C. himalensis*, *C. succinea*, *C. stilbae* on *Picea* (Chen 1984), and possibly *C. tsugae-*

yunnanensis on *Tsuga* (Imazu and Kakishima 1996). In the present study, *C. pirolata* was also observed to have this telial character, although the sterile cells are much shorter than in some of the above-mentioned species. Chen (1984) placed *C. himalensis*, *C. succinea*, and *C. stilbae* in a separate genus, *Stilbechrysomyxa*, based on the stalklike base of the telia. However, there appears to be a gradation in the development of this character in *Chrysomyxa*, and its significance to the relationship among these species is unknown.

Detailed studies of the life cycle and cytology of *C. abietis* have been done by Kursanov (1922), Lindfors (1924), Grill et al. (1978), and Hama (1987). The disease is visible year-round, either by the presence of telia on 1-year-old needles or by the yellowish banding of infected current-year needles (Jørstad 1936). Infection of young needles occurs in early spring by airborne basidiospores liberated from telia. Germ tubes penetrate needles through stomata, producing a monokaryotic intercellular mycelium in the mesophyll (Grill et al. 1978). Immature telia may be visible in late fall, but development is interrupted by winter and resumes the next spring (Jørstad 1936; Grill et al. 1978; Hama 1987). The extent of development in the fall appears to depend on climatic conditions (Lindfors 1924). During telial development, cell fusion occurs at the base of the sorus to produce dikaryotic cells. Subsequent mitotic divisions and septation result in branched dikaryotic hyphae. Karyogamy occurs in the distal cells of these branches and only they can be considered teliospores (Lindfors 1924; Grill et al. 1978). The diploid nucleus migrates into a promycelium that is produced as an extension to a teliospore, meiosis occurs, and then septation to produce a four-celled basidium (Fig. 2.2, C) and four basidiospores. After teliospore germination, the old infected needles are cast. *Chrysomyxa weirii*, the North American microcyclic spruce needle rust, differs from *C. abietis* in its lack of sterile basal cells, its narrower teliospores, dispersal of the teliospores before germination, and the two-celled basidium (see Notes under *C. weirii*; Crane et al. 2000*a*).

Chrysomyxa abietis occurs sporadically depending on climatic conditions. Severe infection of planted spruce has been occasionally reported in Sweden (up to 100% of

needles) (Roll-Hansen 1967), Austria (Donaubauer 1993), Czechoslovakia (Soukup 1994), Norway (Jørstad 1936), and Japan (Takahashi and Saho 1985). Infection increases with increased stand density (Drackov 1965). When combined with other insects or diseases, *C. abietis* may contribute to seedling death (Takahashi and Saho 1985). During several years of observations of the same spruce stands in Sweden, Roll-Hansen (1967) noticed clear differences in level of infection among different provenances. Takahashi and Saho (1985) observed that *P. abies* from Europe was resistant to *C. abietis* in Japan. Therefore it is possible that the rust in Japan is a different race from that occurring in Europe. However, whether infection occurs may depend on flushing time of individual trees rather than on innate resistance.

The observed susceptibility of cultivated American spruces in both Europe and Japan indicates that *C. abietis* could be a serious problem if introduced to North America. Although the fungus was introduced to Kentucky in 1907 on imported *P. abies* from Denmark, it did not become established (Spaulding 1961).

Specimens examined: On *P. abies*. **AUSTRIA:** Ostalpen, Grazer Bergland, Steiermark, Graz-Wenisbuch, an der Wenisbucherstrasse, ± 400 m, 14 May 1975, P. Döbbeler & P. Remler (BPI 1112282, near Type locality). **GERMANY:** Grafrath near Munich, 30 Mar 1939, J.S. Boyce, det. E. Münch (BPI 139689); Oberbayeru, Feb. 1902, E.P. Meinecke (BPI 139702); Eisleben (Prov. Sachsen.), May 1874, G. Winter (BPI 139682). **RUSSIA:** Petropolitana, 5 Jul 1923, A. Jaczewski (BPI 139703). **SWEDEN:** Vasterbotten, 1897, A. Nilsson (BPI 139689); Experimentalfältet prope Stockholm, 10 Jul 1885, J. Eriksson (PUR F545).

Chrysomyxa arctostaphyli Dietel, Bot. Gaz. 19: 303. 1894. **Fig. 2.3, A–I**

≡*Melampsoropsis arctostaphyli* (Dietel) Arthur, N. Am. Flora, Vol. 7, Pt. 2. p. 120. 1907.

=*Peridermium coloradense* (Dietel) Arthur & F. Kern, Bull. Torrey Bot. Club, 33: 426. 1906.

≡*Aecidium coloradense* Dietel, in Engler & Prantl, Natürlichen Pflanzenfam. I: 78. 1897.

=*Peridermium boreale* Arthur & F. Kern, Bull. Torrey Bot. Club, 33: 425. 1906.

Type: On *Arctostaphylos uva-ursi* (L.) Spreng., Three Lakes, Wisconsin, 24 Jun 1892, J.J. Davis 921, comm. Ellis (syntypes PUR 4911! NYBG 53489).

Hosts and distribution: Throughout North America wherever the two host genera, *Picea* and *Arctostaphylos*, occur together. It has been reported in all Canadian provinces and territories, but is more common in the west than the east (Savile 1950; Peterson 1963). Also in Alaska, Oregon, Washington, Colorado, Arizona, Montana, South Dakota, Utah, Wisconsin, Idaho, Wyoming, Michigan, Maine, and New York (Savile 1950; Arthur 1934; Peterson 1963; Ziller 1974). It produces a systemic infection in native North American spruces, including *P. engelmannii*, *P. glauca*, *P. mariana*, *P. pungens*, *P. rubens*, and *P. sitchensis*, and also in ornamental *P. abies*. One report of this rust species from northwestern Russia (Kuprevich and Tranzschel 1957) is unconfirmed. Telia are found on *Arctostaphylos uva-ursi*, *A. patula* Greene, *A. nevadensis* A. Gray, and *A. nevadensis* var. *coloradensis* (Rollins) Harrington.

Description: On *Arctostaphylos*. *Uredinia* unknown. *Telia* on reddish spots that blacken with age, usually hypophyllous, may also be epiphyllous on *A. patula*; gelatinous, in groups, confluent when mature, forming pulvinate crusts, erumpent through epidermis, 0.3–1 mm across. *Teliospores* catenulate, subglobose, cubical, or oblong; 10–19 μm high × 6–16 μm wide. *Basidia* curved, four-celled. *Basidiospores* regular in size and shape, globose to subglobose with a tiny apiculus; 6–8 × 5–7 μm.

On *Picea*. *Spermogonia and aecia* on chlorotic, stunted, current-year needles of perennial witches' brooms. *Spermogonia* amphigenous, numerous, prominent, dark reddish-brown when dry, subepidermal, sometimes arising between epidermis and hypodermis; in cross section, usually ± globose or with a slightly flattened base, 80–160 μm wide × 80–144 μm high. *Spermatia* variable in shape and size, globose, ovoid, or ellipsoidal, 1.6–4.5 × 1.2–2.5 μm. *Aecia* amphigenous, crowded along most of the needle length. *Aeciospores* variable in shape and size, ellipsoidal, ovoid, or polygonal, occasionally globose, subglobose, clavate, or fusiform, often with both ends flattened or with a cap, part of a longitudinal stripe, 16–36 × (12–)14–24 μm, carrot-red (6B7); warts

annulate, irregular in shape, often joined laterally into ridges, basal connections lacking; wall hyaline, very thin (0.8 μm); *wall + warts* 1.6–4.1 μm thick. *Peridium* tubular, dehiscing at apex; outside of cells shallowly concave, smooth; inside of cells shallowly concave, with crowded irregular warts similar to the spores.

Notes: The life cycle of *C. arctostaphyli* was controversial for many years. This species was originally considered to be microcyclic on *Arctostaphylos* (Arthur 1934). Arthur and Kern (1906) established two species of *Peridermium*, *P. coloradense* and *P. boreale*, for the spruce broom rust, based on whether peridial cells overlap, whether infected needles are adherent, and whether spermogonia are prominent. Later, the rust on spruce was thought to be conspecific with *Melampsorella caryophyllacearum* Schroet., the cause of broom rust on *Abies*, because Weir and Hubert (1918) reported experimental infection of *Stellaria calycantha* (Ledeb.) Bong. with aeciospores from brooms on both *Picea* and *Abies*. Later attempts by Pady (1946) and Peterson (1961*a*) to infect *Stellaria* with the spruce rust failed. Hunter (1936) showed that the spermogonial morphology on spruce is more consistent with a *Chrysomyxa* than with *Melampsorella*. Pady (1940, 1942) documented many differences between the morphology of the rusts on the two conifer hosts and maintained that they were different species with the same telial host. The connection between the telia on *Arctostaphylos* and the aecial state, *Peridermium coloradense*, on spruce was finally proven experimentally by Peterson (1961*a*) and confirmed by Ziller (1970).

Chrysomyxa arctostaphyli overwinters as mycelium in the systemically infected brooms on spruce (Fig. 2.3, B) and as mycelium in the leaves of *Arctostaphylos* spp. Telia form on *Arctostaphylos* in early spring (Fig. 2.3, A) and produce basidiospores that cause new infections on spruce, probably through the young needles of newly opened vegetative buds. *Chrysomyxa arctostaphyli* is an exception to the rule that the habit of a *Chrysomyxa* species is similar in both hosts (Savile 1950): the infection is perennial and systemic in the aecial host, but localized and annual in the telial host. The most conspicuous symptoms of spruce broom rust occur in early summer, when yellowish infected needles are present on shoots of witches' brooms. After production of

spermogonia and aecia (Fig. 2.3, C), the needles shrivel and fall off, leaving bare, dead-looking brooms during the winter (Singh 1978). Brooms can occur on the trunk or branches, and after many years attain a diameter of 1 m or more. Trees with up to 41 brooms have been reported in Newfoundland (Singh 1978). Rust brooms are associated with dead tops or branches, bole deformation, and reduced height and diameter growth (Can. For. Serv. 1951, 1952; Peterson 1963; Singh 1978). They may contribute to tree death or to wind breakage when on the trunk (Peterson 1963). Brooms may also provide infection courts for decay fungi such as *Phellinus pini* (Thore:Fr.) Pil. (Mielke and Davidson 1947; USDA 1958; Peterson 1963; Hinds and Hawksworth 1966), render trees unmerchantable, or reduce their aesthetic value in recreation areas (Singh1978). Damage probably depends on the number of brooms per tree, number of years since infection (Peterson 1963), whether on the branch or trunk, and the particular site (Singh 1978). Because *P. abies* is also susceptible, *C. arctostaphyli* is potentially dangerous if introduced to Eurasia, where both host genera are often associated. On the other hand, brooms provide a unique wildlife habitat, serving as food or hiding and nesting habitats for animals. Martens reportedly use them as rest sites all year (Parks and Bull 1997) and flying squirrels use them as den sites (Mowrey and Zasada 1984).

Specimens examined: On *Arctostaphylos patula*. **USA: Utah:** Proctor Canyon E of Hatch, Dixie Natl. Forest, Garfield Co., elev. 4200 ft., 16 Jun 1964, R.S. Peterson (PUR 61363).

On *A. uva-ursi*. **CANADA: Alberta:** 31 km NW of Hinton, highway 40, 27 Jun 1995, P.E.C. (CFB 22018); Hargwen Rd., E of Obed Summit, 26 Jun 1996, P.E.C. (CFB 22033). **British Columbia:** 0.5 mi S Pitts Cr., Kootenay Natl. Park, 3 Jul 1963, J. Petty (CFB 5854). **USA: Montana:** Lost Lk., Glacier Natl. Park, 1 Aug 1924, J.C. Arthur (PUR 4918); Big Fork, 19 Jun 1955, G.B. Cummins (PUR 55334). **Wisconsin:** Three Lakes, 24 Jun 1892, J.J. Davis (PUR 4911, Syntype).

On *P. abies*. **CANADA: Manitoba:** Brandon, 17 Jul 1917, W.P. Fraser (PUR 43461).

On *P. engelmannii*. **USA: Colorado:** Music Pass, Sangre de Cristo Range, Jul 1888, C.H. Demetrio (PUR 43422, TYPE of *Peridermium coloradense*); Lake Eldora, E. Bethel (PUR 43432). **Utah:** Christmas Meadows Campground, Wasatch Natl. Forest, Uinta, 10 Aug 1955,

G.G & R. Solheim (PUR 55359). **Wyoming:** nr. Towner Lk., Medicine Bow Natl. For., 27 Aug 1970, Solheim (PUR).

On *P. glauca*. **CANADA: Alberta:** 31 km N of Hinton, highway 40, 27 Jun 1995, P.E.C. (CFB 22016); E of Obed Summit, highway 16, 26 Jun 1996, Y.H. & P.E.C. (CFB 22026); 20 km E of Hinton, highway 16, 9 Aug 1996, Y.H. & P.E.C. (CFB 22048); Hargwen Rd., E of Obed Summit, 27 May 1998, P.E.C. (CFB 22173).

On *P. mariana*. **CANADA: Quebec:** Seven Islands, Saguenay Co., 10 Aug 1907, C.B. Robinson 845 (PUR 43466).

On *P. pungens*. **USA: Colorado:** S Colorado, Jul 1897, E. Bethel (PUR 43486); N Elk Canyon, Rio Blanco Co., 20 Aug 1902, W.C. Sturgis (PUR 43487). **New Mexico:** Rio Pueblo, 10 Aug 1910, E.O. Wooton (PUR 43493); Santa Fe Natl. For., San Miguel Co., 24 Jul 1937 (PUR 48071).

On *P. rubens*. **USA: Maine:** Isle au Haut, Aug 1910, M. Turner (PUR 43497).

Chrysomyxa cassandrae Tranzschel, Trav. Soc. Nat. St. Petersburg (=Trudi St. Petersb. Obshch. Estestvoisp., Otd. Bot.), Sect. Bot. 23: 28. 1893. **Figs. 2.4, A–K**

=*Uredo cassandrae* Peck & Clinton, in Peck, Ann. Rep. N.Y. State Mus. 30: 54. 1878. [Based on uredinia; specimen bears telia (Savile 1950)]

≡*Chrysomyxa ledi* de Bary var. *cassandrae* (Peck & Clinton) Savile, Can. J. Res. C, 28: 324. 1950. [*nom. nud.*; telia not described]

≡*Melampsoropsis cassandrae* (Peck & Clinton) Arthur, N. Am. Flora, 7: 119. 1907. [telia described]

=*Caeoma cassandrae* Gobi, Scr. Bot. Horti Univ. Petropol. I: 166–180. 1886. [*fide* Tranzschel 1893]

=*Caeoma cassandrae* Rostr., Medd. Bot. For. Kjøbenhavn, 2: 90. 1888. [Based on uredinia]

=*Peridermium consimile* Arthur & Kern, Bull. Torrey Bot. Club, 33: 427. 1906.

Type: On *Chamaedaphne calyculata* (L.) Moench., Levashovo, near St. Petersburg, Russia, 24 May 1892, W. Tranzschel (not seen). The whereabouts of this collection could not be determined. Savile (1955) designated one of Peck's specimens of

Uredo cassandrae as the neotype, based on the presence of telia: on *Chamaedaphne calyculata* (as *Cassandra calyculata* (L.) D. Don), Center, New York, June 1871, Clinton (NYS).

Hosts and distribution: *Chamaedaphne calyculata* is the only telial host of this rust. Reports of *C. cassandrae* on *Andromeda* spp. (*Pieris* spp.) (Arthur 1907; Sydow 1912; Anonymous 1960) are questionable (Jackson 1918; Farr et al. 1989). They appear to have originated with Arthur (1907), but were later corrected (Arthur 1925). *Pucciniastrum myrtilli* Arthur (*Uredo andromedae* Cooke), which has echinulate urediniospores (Jackson 1918), was likely misidentified as a chrysomyxa. *Chrysomyxa cassandrae* occurs throughout the North Temperate Zone wherever *C. calyculata* occurs, independent of host alternation. This includes northern Canada (all provinces and territories), and the United States (Alaska, and eastern states south to Pennsylvania), northern Europe (Denmark, Sweden, Finland, Lithuania, Russia (Karelia, Kamchatka, Sakhalin)), and Japan. It has not been reported on spruce in Japan (Hiratsuka et al. 1992).

Aecial hosts of *C. cassandrae* are *Picea abies*, *P. mariana*, *P. pungens*, and *P. glauca*. This study extends the range on spruce to western Canada. In addition to field collections on both hosts in central Alberta, telia and/or uredinia were found on *C. calyculata* in vascular plant collections (CAFB and ALTA) from northern Alberta, Saskatchewan, and Manitoba. Therefore, it is probably much more common on spruce than previously thought, but it could be mistaken for *C. nagodhii*. It could also be overlooked on *C. calyculata*, which is a small inconspicuous shrub that occurs much more sporadically than *L. groenlandicum*; it is characteristic of black spruce peatlands and flooded wetlands (Zoladeski et al. 1995; Beckingham and Archibald 1996).

Description: On *Chamaedaphne*. *Uredinia and telia* hypophyllous on leaves of previous year, uredinia occasionally on petiole or main leaf vein; causing brown leaf spot. *Uredinia* in groups, discrete, separate from or forming in telia, circular, 1/4–3/4 mm in diam, subepidermal, or when on petiole, arising beneath several layers of collenchyma cells; having an inconspicuous peridium of collapsed, thin-walled cells. *Urediniospores* ellipsoidal, ovoid, lenticular, or occasionally subglobose, with a broad cap at one or both

ends, part of a broad flat longitudinal smoother area with a broken, skirtlike edge, 16–33 × 14–24 μm, deep orange (6A8); warts crowded, cylindrical, annulate, with flat tops, except in longitudinal smoother area, where they are broad, shallow bumps; wall hyaline; *wall + warts* 1.2–2.5 μm thick. *Telia* in clusters of irregular size and shape, pulvinate, erumpent through epidermis, mandarin orange (6B8). *Teliospores* cuboid to oblong, 12.5–26 × 12.5–18 μm, wall thin, < 1 μm. *Basidiospores* globose, subglobose, or irregular, 9–10(–12) × 8–10(–12) μm, surrounded by a mucilaginous sheath when deposited on microscope slides.

On *Picea*. *Spermogonia and aecia* on current-year needles, causing premature needle loss. *Spermogonia* amphigenous, prominent, subepidermal, 105–170 μm wide × 86 – 160 μm high, rusty orange or brown when dry; hymenium poorly defined, flat to concave. *Spermatia* ellipsoidal, 2.5–3 × 1.5–2 μm. *Aecia* discrete, not confluent, tubular or tonguelike, even in width, 1/4–1/2 mm, up to 3 mm long, mostly epiphyllous. *Aeciospores* globose, subglobose, ellipsoidal, or slightly ovoid, with a broad shallow cap at one or both ends, part of a broad longitudinal smoother area with a broken, skirtlike edge, 17–31 (–36) × 14–22 μm, orange (6A7); warts cylindrical with thin basal connections, annulate, with flat or uneven tops, except in longitudinal smoother area, where they are broad, shallow bumps; wall hyaline, 0.8 μm thick; *wall + warts* 0.8–3.3 μm thick. *Peridium* persistent; cells overlapping, polygonal, round or square, outer surface shallowly concave, wall smooth to slightly rough, inner surface flat or convex and covered densely with fingerlike warts, side walls narrow and striate or rugulose.

Notes: Like most telial hosts of *Chrysomyxa*, *C. calyculata* has evergreen leaves in which the mycelium of *C. cassandrae* overwinters. In early spring, telia form on the underside of the leaves, followed by uredinia (Fig. 2.4, A, B). In Europe, telia may be rare (Sydow 1912; Kuprevich and Tranzschel 1957), but this does not seem to be the case in North America, where telia were seen in many herbarium collections from diverse locations and on infected plants in the field. Basidiospores produced by the germinating teliospores cause infection on young tender needles of adjacent spruce. Abundant spermogonia form on the needles, followed by aecia (Fig. 2.4, C), which later result in

premature defoliation. Both aeciospores and urediniospores cause new infections on *C. calyculata*.

Inoculation experiments by Clinton (1908) and Fraser (1911, 1912) showed the connection of the the aecial state, *Peridermium consimile*, on *Picea mariana* and *P. rubens*, with the uredinia and telia on *C. cassandrae*. In the present study, *P. glauca* and *P. pungens* were inoculated with basidiospores from infected *C. calyculata* from north-central Alberta. These samples, in addition to Fraser's (PUR) from Nova Scotia, provided authentic material to formulate an accurate description of the spermogonia and aecia of *C. cassandrae*. The telial host and the urediniospore morphology of *C. cassandrae* are distinct from other members of the *C. ledi* complex, as defined by Savile (1950, 1955). The mucilaginous sheath around the basidiospores of *C. cassandrae* and their large size and irregular shape are also different from other members of the *C. ledi* complex. Obscuring these differences by placing *C. cassandrae* as a variety of the European *C. ledi* (Savile 1950, 1955) is not supported. The aeciospores of *C. cassandrae* and *C. nagodhii* are, however, difficult to distinguish, even with SEM (Figs. 2.4, 2.13), and the broadleaved hosts of both rusts, *Ledum groenlandicum* and *C. calyculata*, may occur in the same boggy habitats. Outbreaks of spruce needle rusts in such areas will likely be a combination of both *C. cassandrae* and *C. nagodhii* as well as the epiphyllous *Ledum* rust, *C. ledicola* (Lindgren 1933). The subtle differences listed in Table 2.1 may aid in distinguishing between the aecia of *C. nagodhii* and *C. cassandrae*. In Eurasia, the larger spore size, fine tapering warts, and narrow longitudinal groove of *C. ledi* aeciospores should be adequate characters to distinguish this species from *C. cassandrae* (see mean urediniospore and aeciospore size, Fig. 2.9, A and B).

Chrysomyxa cassandrae is common on spruce in the northeastern United States, where it has been reported to cause severe epidemics of needle rust on *P. mariana* and *P. pungens* (Lindgren 1933; Hodson and Christensen 1942). In this study, artificial inoculation also produced severe infection of current-year needles on 8-year-old *P. pungens* (Fig. 2.4, C), supporting previous evidence that this species is highly susceptible and may be at risk if grown in areas where *C. calyculata* is abundant.

Table 2.1. Comparison of the aecial stage of *C. nagodhii* and *C. cassandrae*

	C. nagodhii	*C. cassandrae*
Aeciospore ornamentation	Broad caplike longitudinal stripe	Broad caplike longitudinal stripe
	Cap slightly rough with smooth edge	Cap with broad flat warts; edge broken and fissured
	Warts tapered, 4 annuli	Warts cylindrical, 3 annuli
Outer peridium surface	Cells deeply concave, with sharply defined edges, slightly rough surface	Cells shallowly concave with poorly defined edges, smooth to slightly rough surface
Inner peridium surface	Cells shallowly concave with a raised edge; warts shallow, irregular, discrete and fine or forming shallow ridges, sometimes appearing reticulate	Cells flat to convex; warts distinct and densely crowded, appear fingerlike; edges striate or rugulose

Specimens examined: On *Chamaedaphne calyculata.* **CANADA: Alberta:** Goose Mtn. Fire Tower Rd., 10 km NW of Swan Hills, 28 Jun 1981, J.D. Johnson (CAFB 811457); 13.3 km N of Calling L., hwy. 813, 13 Jun 1997, Y.H. et al. (CFB 22069, 22156). **British Columbia:** Fort Nelson, 20 Jul 1966, D.G. Lund & J. Grant (DAVFP 17131). **Manitoba:** Berens River, 29 Jul 1933, G.R. Bisby (PUR 44378). **Northwest Territories:** Indin L., District of Mackenzie, 64°17' N, 115°12' W, 12 Aug 1949, W.J. Cody & B.J. McCanse (PUR 52049). **Nova Scotia:** Pictou, 13 Jun 1910, W.P. Fraser (PUR 4847). **Yukon Territory:** 3/4 mi NE of Mayo, 2 Aug 1949, J.A. Calder et al. (PUR 52026). **FINLAND:** Karelia, 18 Aug 1899, J.I. Lindroth (PUR 511). **RUSSIA:** Shisuka, S. Saghalien [Sakhalin], 12 Aug 1928, N. Hiratsuka (PUR F12673); Aihama, S. Saghalien, 17 Jul 1927, N. Hiratsuka (PUR F12674). **USA: Wisconsin:** Hope L. bog nr. Cambridge, Jefferson Co., 12 Jun 1965, H.C. Greene (PUR 61510).

On *Picea glauca.* **CANADA: Alberta:** Edmonton, 19 Jul 1997, P.E.C. (CFB 22128, from inoculation).

On *P. mariana.* **CANADA: Alberta:** 13.3 km N of Calling L, hwy. 813, 13 Jun 1997, P.E.C. et al. (CFB 22118, 22132). **Nova Scotia:** Pictou, 24 Jul 1910, W.P. Fraser (PUR 4809). **USA: Maine:** Kittery Pt., Jul [no year], R. Thaxter (PUR 4798). **New York:** Junius Swamp, Jul 1905, E.J. Durand (PUR 4800).

On *P. pungens.* **CANADA: Alberta:** Edmonton, 19 Jul 1997, P.E.C. (CFB 22126, from inoculation). **USA: Wisconsin:** Jul 1941, E.L. Chambers (PUR 49816).

On *P. rubens.* **CANADA: Ontario:** Sparrow L., 26 Aug 1907, J.C. Arthur (PUR 4820). **Nova Scotia:** Pictou, 24 Jul 1910, W.P. Fraser (PUR 4824, from inoculation).

C. chiogenis Dietel, Bot. Gaz. 19: 303. 1894. **Fig. 2.5, A–J**

≡*Melampsoropsis chiogenis* (Dietel) Arthur, N. Am. Flora, 7(2): 121. 1907.

Type: On *Gaultheria hispidula* (L.) Muhl. (as *Chiogenes serpyllifolia* Salisb.), Forest Co., Wisconsin, June 1893, J.J. Davis 6078, comm. Ellis (PUR 4899! Isotypes BPI 190498, NY 53850, 27734).

Hosts and distribution: Telia and uredinia have been collected on *G. hispidula* in British Columbia, eastern Canada (Ontario, Quebec, New Brunswick, Newfoundland), and northeastern USA (New York, New Hampshire, Wisconsin). It likely has a much

wider distribution. In the aecial state it is known only from inoculations conducted in Ontario on current-year needles of *Picea mariana* and *P. glauca* (Faull 1936).

Description: On *Gaultheria*. *Uredinia and telia* hypophyllous on leaves of previous year. *Uredinia* scattered, circular to elliptical, 0.2–0.8 mm in diam, subepidermal, dehiscent from center, covered by a peridium of one or more layers of collapsed cells with walls 1.2–1.6 μm thick. *Urediniospores* globose, subglobose, or ellipsoidal, with a flat area, 15–32 × 13–21 μm; warts cylindrical or joined to form elongated or mazelike ridges, annulate; wall hyaline, very thin; *wall + warts* 1.2–2.5 μm thick. *Telia* hypophyllous, subepidermal, orange, waxy, scattered, round or oval, 0.2–1 mm in diam. *Teliospores* catenulate, six or more in a chain, 8–19 × 7–15 μm. *Basidia* slightly curved to strongly arched, 4-celled. *Basidiospores* subglobose, 7–9 μm in diam (Faull 1936).

On *Picea*. *Spermogonia* unknown. *Aecia* on discolored portions of current-year needles, along stomatal lines, amphigenous, 0.5–2 mm wide. *Aeciospores* ellipsoidal or ovoid, ends smooth, (15)20–28 × 13–18 μm; warts annulate, broad and truncate with uneven tops, often joined into ridges; wall hyaline, very thin; *wall + warts* 2–3.3 μm thick. *Peridium* delicate, shredding at apex, cells separate easily, densely warted inside, smooth outside.

Notes: *Chrysomyxa chiogenis* likely overwinters in the evergreen leaves of its telial host, then produces basidiospores in early spring, which infect young spruce needles. An early report that this rust might be systemic in *Gaultheria* (Savile 1950) was based on misinterpretation of heavy localized infections (Savile 1955). This rust is rarely collected, probably because of the inconspicuous nature of the telial host. The natural distribution of the aecial stage is unknown because the aeciospores are similar in size to species that have been considered part of the *C. ledi* complex (*sensu* Savile 1950, 1955) (Fig. 2.9). In specimens examined from both Quebec and British Columbia, telia were abundant; therefore the aecial stage may be more common than thought.

Inoculated material by Faull (1936) and his colleagues was used to formulate the description of the aecial state. Two of these collections, however, are questionable

because there were spermogonia present on the needles. It is possible that there was contamination from other rusts in the vicinity (some of the experiments were conducted outdoors) or that the absence of spermogonia is not a reliable feature. However, the former possibility is more likely, because the spores of the doubtful collections (BPI 190502 and 190503) were morphologically different from those found on needles without spermogonia, and an infected control was also included in the material.

In spite of its elusive nature, *C. chiogenis* is interesting for several reasons. The absence of spermogonia is unusual within the genus and suggests that some modification has occurred to its sexual cycle. Four rust specimens (identified as *C. ledi* in DAVFP) on *Picea sitchensis* from coastal British Columbia (Queen Charlotte Islands and Vancouver Island) also lack spermogonia and may be this species. However, *G. hispidula* is not known from the Queen Charlotte Islands (Calder and Taylor 1968) and two of these samples have aeciospores much longer (up to 44 μm) than Faull's inoculated material, making it unlikely that they are *C. chiogenis* (see further discussion under *C. vaccinii* and *Peridermium zilleri*). Ziller noted that the urediniospores of *C. vaccinii* are almost indistinguishable from those of *C. chiogenis*. This study shows that the spores of *C. vaccinii* are more consistently elongated than those of *C. chiogenis*, and the warts of *C. vaccinii* urediniospores are narrower and more discrete. Apart from these differences, they are very similar in morphology, including the presence of an indistinct smoother area on most urediniospores.

Also intriguing is the very different morphology of the urediniospores of *C. chiogenis* from eastern (Quebec) and western (British Columbia) Canada (Fig. 2.5, C, D). In the samples from British Columbia, spores are longer (max. 32, compared with 25 μm), the warts are well-defined and joined into long ridges at the spore ends and sometimes elsewhere; wart tops are bumpy and pitted (Fig. 2.5, D, E). From Quebec, the spores are more rounded, warts are smoother-topped, basal connections between warts are less well-developed, and spores have a rough longitudinal stripe (Fig. 2.5, C). Although they lack a reticulate area, some urediniospores from Quebec closely resemble *C. reticulata* in size, shape, and overall wart morphology. Examination of more collections

is needed to determine whether eastern and western collections differ consistently. It should also be noted that published reports of the urediniospore size are extremely variable, from a maximum length of 29 μm (Dietel 1894) to 42 μm (Savile 1950).

Specimens examined: On *Gaultheria hispidula*. **CANADA: British Columbia:** Prince George, 4 Sep 1950, W.G.Z. (DAVFP 6047); Wells, 20 Jul 1954, J. Grant & C.B. Cottrell, (DAVFP 9388); Prince George, 28 Jun 1966, J. Grant (DAVFP 17135). **Quebec:** Mi 13, Clova, 24 Jun 1931, C.G. Riley (WINF(M) 1793). **USA: Wisconsin:** (host as *Chiogenes serpyllifolia*) Forest Co., Jun 1893, J.J. Davis (PUR 4899, Type).

On *Picea glauca* (from inoculation). **CANADA: Ontario:** Bear Is., L. Timagami, 1928, G.D. Darker 2321 (BPI 0190499), 7 Aug 1924 (BPI 190501); 12 Sep 1924, W.R. Watson (BPI 190502, 190503).

Chrysomyxa empetri J. Schröt. ex Cummins, Mycologia, 48: 602. 1956. **Figs. 2.6, A–J**

=*Chrysomyxa empetri* (Pers.) J. Schröt., in Cohn's Kryptogamen-Flora von Schlesien, III(1): 372. 1887. [*nom. nud.*; based on uredinia]

=*Chrysomyxa empetri* J. Schröt. ex Jørst., K. Nor. Vidensk. Selsk. Skr. 1935, 38: 51. 1936. [Based on telia, but no Latin description]

=*Chrysomyxa empetri* J. Schröt. ex Faull, J. Arnold Arbor. 18: 141–148. 1937. [Based on telia, but no Latin description]

=*Uredo empetri* Pers. ex DC. in Flore Fr. 6: 87. 1815.

≡*Chrysomyxa empetri* (Pers.) Rostr., Medd. Grönl., Kjøbenhavn, III: 536. 1888. [*nom. nud.*; telia mentioned, but not described]

≡*Melampsoropsis empetri* (Pers.) Arthur, N. Am. Flora, 7: 118. [Based on uredinia]

≡*Caeoma empetri* (Pers.) Link, in Willd. Spec. Plant. II: 16, 1825.

≡*Thekopsora empetri* (Pers.) P. Karst., Bidr. Känned. Finlands Nat. Folk, 31:143. 1879.

=*Erysibe empetri* Wallr., Flora Cryptogam. Ger. II: 199, No. 1621. 1833.

Type: On *Empetrum nigrum* L., Kongsvoll in Opdal, Sør-Trøndelag, Norway, Jul 1887, A. Blytt, comm. I. Jørstad, Botanical Museum, Oslo (Isotype BPI 191189!).

Hosts and distribution: The aecial stage of *C. empetri* is rare but has been documented on *Picea engelmannii*, *P. glauca*, *P. abies*, *P. sitchensis*, and *P. rubens* (Faull 1937). It is unknown in Japan and Britain. Uredinia and rarely telia occur on previous year's foliage of *Empetrum nigrum*, *E. eamesii* Fern. & Wieg., *E. hermaphroditum* (Lange) Hagerup, and *E. rubrum* Vahl (Faull 1937; Roll-Hansen and Roll-Hansen 1995). Telia have been found in Quebec, Greenland, Norway, New Hampshire, and Japan. The uredinial stage is found in the entire Northern Hemisphere and the Falkland Is., far beyond the limits of spruce.

Description: On *Empetrum*. *Uredinia and telia* mostly on abaxial surface (recurved margins) of overwintered leaves, uredinia occasionally on adaxial surface. *Uredinia* erumpent through the epidermis, elliptical or elongated, 0.5–2 mm long, 1 to several per leaf, with a peridium consisting of one layer of thin-walled (1 μm) oblong cells that remain attached to the underside of the epidermis after it splits. *Urediniospores* catenulate, ovoid or ellipsoidal, occasionally subglobose or globose, with one end flat or with a small notch, part of a narrow or broad, punctate longitudinal cap, deep orange (6A8), 23–46 × 19–36 μm; warts fine, even in size and shape, tapering slightly, annulate, with fine, but complex basal connections; *wall + warts* variable, 1.6–3.3(–4.1) μm. *Telia* elongated, conspicuous, separate or confluent, pulvinate, waxy when young, velvety when mature, subepidermal in origin. *Teliospores* catenulate, oblong or cuboid, 18–45 × 15–24 μm (Jørstad 1936). *Basidiospores* globose to subglobose with a small apiculus, 8–10 × 7–9 μm.

On *Picea*. *Spermogonia and aecia* on current-year needles. *Spermogonia* numerous, amphigenous, slightly raised, prominent, round to slightly elongated on needle surface, almost black when old, subepidermal, 120–200 μm wide × 100–150 μm high, in cross section almost triangular in shape, hymenium flat to slightly concave. *Spermatia* globose to ellipsoidal or ovoid, dark rusty-brown, ovoid, ellipsoidal, or subglobose, 4.1–5.7 × 2.5–4.1 μm. *Aecia* amphigenous, discrete, tonguelike, 0.3–1.4 mm wide, about

0.5 mm high. *Aeciospores* ellipsoidal or ovoid, or lenticular, often quite narrow and elongated, 27–50(–60) × 16–37 μm, with one or both ends flat or with a delicate cap, part of a broad punctate longitudinal cap with an irregular edge; warts tiny, tapering slightly, discrete, annulate, with complex basal connections; *wall + warts* 1.2–3.3(–4.1) μm. *Peridium* dehisces irregularly at apex, cells often in horizontal rows, square or polygonal, overlapping slightly; outer cell surface deeply concave, smooth or punctate, thin-walled; inner cell surface covered with shallow dense warts that are often confluent, forming elongated ridges.

Notes: *Chrysomyxa empetri* is very common in its uredinial stage, probably occurring over the entire range of its *Empetrum* hosts. DeCandolle (1815) described its uredinia from specimens distributed 2 years earlier in Mougeot and Nestler's Stirpes Cryptogamae Vogeso-Rhenanae and to which Persoon had attached the name *Uredo empetri* (Faull 1937). Schröter (1887) referred the rust to *Chrysomyxa*, as did Rostrup (1888). Both Rostrup (1888) and Lagerheim (1893) found telia in Greenland, but neither described this stage. Telia were first described by Jørstad (1936) from material collected by Axel Blytt in July 1887, in Norway. The following year, Faull described telia from North America (Quebec). Neither of these early descriptions was in Latin. Cummins (1956) provided the necessary Latin diagnosis to comply with the International Code of Botanical Nomenclature (1952 and later editions).

Chrysomyxa empetri overwinters as mycelium in the evergreen leaves of *Empetrum* spp., and can survive without its aecial host, spruce. Where telia occur (Fig. 2.6, B), they are the first sori to appear in late spring or early summer. They are active for about 2 weeks, after which the leaves bearing them wither, and the sori become unrecognizable (Faull 1937). Throughout the summer only the uredinia are apparent on the leaves (Fig. 2.6, A).

Faull (1937) proved the macrocyclic life cycle of this rust with extensive inoculation experiments both of *Picea* and *Empetrum*. Records of this rust on spruce have been considered dubious because of the similarity in spore size to *C. ledicola* (Savile 1950). However, careful examination of Faull's inoculated spruce has shown that there

are several features that, in combination, can provide a reliable diagnosis (Table 2.2). Based on these criteria, several specimens reportedly of *C. empetri* on *Picea* were found to be *C. ledicola*, including two of Savile's collections on needles and cones from Great Whale River (CFB 8764, ex DAOM 23437; PUR 52097, ex DAOM 23438) and the only reports on *Picea* spp. from Alberta (CFB 320 and 5743). One collection from Dal Lake, N.W.T., was re-identified as *Pucciniastrum* sp. Therefore, we cannot assume, as did Savile (1950), that the aecial state is common. Although this rust has frequently been collected on *Empetrum*, telia have rarely been reported. Young uredinia may be mistaken for telia because of their gelatinous appearance, but in cross section the ornamented surface of immature urediniospores is visible. Genuine telia, when mature, are easily recognizable by their velvety appearance (Fig. 2.6, B, top).

Specimens examined: On *E. hermaphroditum*. **SWEDEN:** Torne Lappmark, Jukkasjärvi Parish, Abisko, close to "Abisko Naturvetenskapliga Station", 17 Jul 1952, L. Holm (1006) (PUR F17066); Ångermanland, Nordmaling Parish, Järnäsklubb (no date), S.O. Björkman (K (Flora Suecica 2663)).

On *E. nigrum*. **CANADA: Alberta:** 20 km SE of Grande Cache, 27 Jun 1995, P.E.C. (CFB 22012); 50 km N of Hinton, Hwy 40, 8 Aug 1996, P.E.C.& Y.H. (CFB 22062). **Northwest Territories:** Chesterfield Inlet, Keewatin Dist., 6 Aug 1950, D.B.O.S. & C.T. Watts (ex DAOM 25764, PUR 53200). **Quebec:** Métis Beach, 20 Jun 1935, J.H. Faull (ex Herb. Faull, PUR 47904). **FINLAND:** W of Kemijärvi, 7 Aug 1998, P.E.C. (CFB 22192). **JAPAN:** Mt. Shirane (Nikko), Prov. Shimotsuke, 8 Aug 1930, N. Hiratsuka (ex Herb. Hiratsuka 417, PUR F12678). **NORWAY:** Sør-Trøndelag, Kongsvoll in Opdal, Jul 1887, Axel Blytt, det. I. Jørstad (ex Herb. Faull, BPI 191189, Isotype). **RUSSIA:** Contact (61°55' N, 147°50' E), Magadan Region, 20 Jul 1994, M. Kakishima, Y. Ono, S. Kaneko, M. Imazu (TSH R9256). **USA: Alaska:** McKinley Natl. Park, 29 Jun 1928, Ynes Mexia (2061) (BPI 191187E). On *P. glauca*. **CANADA: Newfoundland:** Heart's Content, 21 Aug 1954, W.C. Parrott (CFB 7809, ex DAVFP 9376). **Quebec:** Métis Beach, 24 Aug 1935, J.H. Faull (PUR 47902, ex Herb. Faull, from inoculation).

On *P. rubens*. **USA: Maine:** Mt. Desert Is., Hancock Co., 29 Jul 1939, A.E. Prince (PUR 51605).

Table 2.2. Comparison of *C. empetri* and *C. ledicola* on spruce

	C. empetri	*C. ledicola*
Aecia	Discrete, tonguelike, up to 1.4 mm	Discrete or confluent, variable in width, up to 4 mm
Aeciospores		
Ornamentation	Cap at one or both ends, part of a broad shallowly warted smoother area; warts tiny, discrete; annuli difficult to see by LM	No cap or smooth area, sometimes an inconspicuous longitudinal indentation; warts large, coarse; annuli obvious by LM
Shape, size (μm)	Ovoid to ellipsoidal, often very narrow and elongated; 27–50(–60) × 16–37	Globose to subglobose, sometimes elongated; 23–60 × 18–54
Wall	Thin, difficult to distinguish from warts	Thick (up to 2.5 μm), easy to distinguish from warts
Peridium	Persistent, dehisces irregularly at apex	Evanescent, forming a small fringe around base of mature aecium
Inside of cells	Narrow warted margin, poorly defined; densely warted elsewhere, sometimes in ridges	Well-defined, coarsely striate lateral walls; very fine shallow warts, sometimes in undulating rows
Outside of cells	Deeply concave, smooth or punctate	Concave, with broad side-walls, rugulose
Spermogonia	120–200 μm wide, 100–150 μm high; hymenium flat to slightly concave	100–200 μm wide, 90–150 μm high; hymenium slightly to strongly concave
Spermatia	4.1–5.7 × 2.5–4.1 μm	2–4.1 × 1.6–3.3 μm

Chrysomyxa ilicina (Arthur) Arthur, Man. Rusts U.S. Can., p. 31. 1934. **Fig. 2.7, A–G**

=*Melampsoropsis ilicina* (Ellis & Everh.) Arth., N. Am. Flora, 7: 688. 1925. [telia described]

≡*Aecidium ilicinum* Ellis & Everh., Bull. Torrey Bot. Club, 24: 284.1897. [Based on uredinia]

Type: On *Ilex opaca* Ait., Fayette Co., West Virginia, 23 Apr 1896, L.W. Nuttall 839 (PUR 4596! as *Aecidium ilicinum*). Unfortunately, this specimen no longer contains telia, but detailed drawings of telia by F.D. Kern accompany the specimen and therefore it can still serve as the type. BPI 190530, marked as "part of type," also lacks telia.

Hosts and distribution: Known only on its telial host, *Ilex opaca*, from a few locations in central West Virginia, Tennessee, Texas, and North Carolina.

Description: On *Ilex*. *Uredinia and telia* hypophyllous, causing leaf spot. *Uredinia* in groups or scattered, circular, elongated, or irregular in shape, 1/4–1 mm in diam, arising within the telia or separately, erumpent through the epidermis. *Urediniospores* elongated, ellipsoidal or irregular, with one flat side or end, 20–33 × 14–23 μm; one-half or more of spore is covered with a broad, longitudinal smoother area with shallow bumps or ridges; wall hyaline, very thin, 0.4–0.8 μm; warts annulate, cylindrical or tapering, irregular in size and shape, sometimes joined laterally, basal connections thin or absent; *wall + warts* 2.5–4.1 μm thick. *Telia* similar in gross appearance to uredinia. *Teliospores* catenulate, possibly consisting of two subglobose cells, 23–32 × 13–23 μm (Arthur 1934), wall thin (1 μm), smooth.

Notes: Little is known about *C. ilicina*. Most of the herbarium material examined was of poor condition and telia were scarce. The urediniospores closely resemble other *Chrysomyxa* species, especially *C. cassandrae* and *C. chiogenis*, in their size and surface morphology. Arthur (1934) stated that the generic assignment was somewhat uncertain, probably because the telial host is in the Aquifoliaceae (holly family) rather than in the Ericaceae. The classification of the host family is controversial, but no indication of a close relationship to Ericaceae could be found in the literature (Cronquist 1988; Anderberg 1992, 1993; Cuénod et al. 2000). *Ilex opaca*, however, is similar to many

ericaceous plants in having evergreen leaves, permitting overwinter survival of the rust mycelium. The possibility that the teliospores are two-celled (Fig. 2.7, C, D), as observed in the one specimen with adequate numbers of telia to permit sectioning, casts doubt on the placement of this rust in *Chrysomyxa*. However, the single septum was seen only in the uppermost cells of the telium, not basally. Therefore this condition may also be the start of basidium formation. Study of the ontogeny is needed. Inoculation experiments to determine the aecial host and examination of more telial collections will be necessary to answer these questions.

Specimens examined: On *Ilex* sp. **USA: North Carolina:** Mocksville, 29 Sep 1941, W.B. Wood (BPI 190527). **Tennessee:** Signal Mt., Hamilton Co., 27 May 1934, A.J. Sharp (BPI 190531, PUR 44345); Smith Bros. Nursery, McMinnville, 20 May 1955, A.E. Straby (BPI 190533); Shady Valley, Johnson Co., 17 Jun 1934, A.J. Sharp (BPI 190532); 20 May 1934 (BPI 190534, PUR 44346). **Texas:** Waxahachie, 2 May 1949, F.J. Inman (BPI 190526). **West Virginia:** Fayette, Apr 1896, L.W. Nuttall (BPI 190529); Short Cr., Fayette Co., Apr 1896, L.W. Nuttall (BPI 190530); Nuttallsburg, Short Cr., 16 Jul 1930, C.R. Orton (BPI 198696, PUR 44343).

Chrysomyxa ledi de Bary, Bot. Z. 37: 809. 1879. **Fig. 2.8, A–H**

≡*Chrysomyxa ledi* de Bary var. *ledi* Savile, Can. J. Res. C, 28: 324. 1950.

=*Melampsoropsis abietina* (Alb. & Schwein.) Arthur, N. Am. Flora, 7: 119. 1907. *p.p.*

=*Uredo ledi* Alb. & Schwein., Consp. Fung., p. 125. 1805.

≡*Erysibe ledi* (Alb. & Schwein.) Wallr., Flora Cryptogam. Ger. II, No. 1620, p. 199. 1833.

≡*Coleosporium ledi* (Alb. & Schwein.) Schröt., in Cohn Beitr. Biol. Pflanzen, III(1): 55. 1879.

=*Caeoma ledi* Link, Willdenow, Spec. Plant. Gen. VI, 2, p. 15. 1825. [*fide* Saccardo 1888]

=*Caeoma ledi* Schltdl., Flora Berol. II: 122. 1824.

=*Caeoma piceatum* Link, in Linn. Spec. Plant. II, p. 62–63. 1825. *p.p.*

=*Uredo ovoideo-aurantiaca* Bonord., Coniom. Cryptom., p. 32. 1860. [*fide* Saccardo 1888]

=*Uredo abietina* Spreng., Syst. Veg. IV, p. 572. 1827. [*fide* Thümen 1880]

=*Pucciniastrum ledi* Karst., Bidr. Känned. Finlands Nat. Folk, 31: 57. 1879. [*fide* Sydow 1915]

=*Aecidium abietinum* Alb. & Schwein., Consp. Fung., p. 120. 1805. *p.p.*

≡*Peridermium abietinum* (Alb. & Schwein.) Thüm., Mitt. Forstl. Versuchswesen Oesterr. 2: 320–321. 1880.

Type: On *Ledum palustre* L., Grunewald near Berlin, Germany.

Hosts and distribution: *Chrysomyxa ledi* occurs in Eurasia throughout the range of its broadleaved hosts, independent of host alternation: in northern Europe, on *Ledum palustre*; in Siberia, on *L. hypoleucum* Kom. and *L. macrophyllum* Tolm. (Azbukina 1974); and in Japan, on *Ledum palustre* ssp. *diversipilosum* Hara (Hiratsuka et al. 1992). The aecial stage is found on native and ornamental spruces: in Europe, on *Picea abies*, *P. engelmannii*, *P. glauca*, *P. obovata* Ledebour, *P. mariana*, and probably others (Hylander et al. 1953; Kuprevich and Tranzschel 1957; Gjaerum 1974; R. Jalkanen, Finnish Forest Research Inst., pers. comm.); in Siberia, on *P. ajanensis* Fisch. (Azbukina 1974); in China, on *P. likiangensis* (Franchet) Pritzel (Spaulding 1961); and in Japan, on *P. jezoensis* (Hiratsuka et al. 1992) and *P. glehnii* (Ono and Isono 1992).

Description: On *Ledum*. *Uredinia and telia* hypophyllous on leaves of previous year, uredinia occasionally caulicolous. *Uredinia* circular, 1/4–1/3 mm wide, single or in groups, with a peridium of 2–3 layers of thin-walled pseudoparenchymatous cells that are much smaller than spores. *Urediniospores* globose, subglobose, or ovoid, occasionally ellipsoidal, 18–30 × 16–26 μm, sometimes notched or flattened at one end because of a narrow longitudinal groove with or without a well-defined edge; wall hyaline, 0.5–0.8 μm thick; *wall + warts* 2.5–2.9 μm thick. *Telia* sparsely aggregated, flat, blood-red to orange-red. *Teliospores* readily separating, oblong to cuboid, 13–30 × 10–20 μm

(Saccardo 1888; Sydow 1915; Kuprevich and Tranzschel 1957). *Basidiospores* ovoid, 11 × 7 μm (Saccardo 1888).

On *Picea*. *Spermogonia and aecia* on current-year needles. *Spermogonia* amphigenous, subepidermal; in cross section, hymenium concave to slightly flattened, 100–190 μm wide × 90–150 μm high. *Spermatia* ovoid, subglobose, or globose, 1.2–2 × 1.2–1.6 μm. *Aecia* amphigenous, tubular, 1/4–1 1/4 mm wide. *Aeciospores* ovoid, ellipsoidal, globose, or subglobose, 20–38 × 15–28 μm, with a distinct narrow longitudinal groove; warts crowded, annulate, tapering; wall hyaline, 0.8 μm thick; *wall + warts* 1.6–4.9 μm thick. *Peridium* dehiscing at apex, later shredding, leaving a fringe around sorus; outside of cells deeply concave, ± smooth; inside of cells shallowly concave, shallowly and densely warted, warts often arranged in undulating rows; lateral margins broad (3–6 μm or more) with coarse striations.

Notes: *Chrysomyxa ledi*, the Eurasian hypophyllous *Ledum* rust, is distinct in morphology from the rusts occurring on *Ledum* in North America. Historically the North American species have been considered conspecific with *C. ledi* (Arthur and Kern 1906; Arthur 1907, 1962; Boyce 1943) or as varieties of the latter (Savile 1950, 1955). Scanning electron microscopy clearly demonstrates the morphological differences, especially of the aeciospores and urediniospores, distinguishing *C. ledi s.s.* from the North American species, described herein as *C. nagodhii* and *C. neoglandulosi* (see Notes and figures under those species). The aeciospores of *C. ledi* are also somewhat larger than the aeciospores of the North American species (Fig. 2.9). Five specimens from Japan (PUR) were examined on several varieties of *L. palustre* (now all considered *L. palustre* ssp. *diversipilosum* Hara (Hiratsuka et al. 1992)); they also differ somewhat from the European specimens, and therefore were not used to compile the species description. Their urediniospores are smaller (14–25 × 11–21 μm) than the European samples and their surface ornamentation variable (Figs. 2.8, B, D). Warts have broad irregular tops, and spores may have a flattened end where warts are confluent; however, there is seldom a well-defined groove. A thorough investigation of the hypophyllous rusts on *Ledum* and *Rhododendron* in Japan is needed to determine whether they should be considered

separate species from rusts on the same host genera elsewhere (see also Notes under *C. rhododendri*).

De Bary (1879) proved experimentally the connection between the telial stage of *C. ledi* on *Ledum palustre* and the aecial stage on spruce, and detailed the differences between *C. ledi* and *C. rhododendri*. The coarser margins of the peridial cells of *C. ledi* (Fig. 2.8, H) are an important distinguishing feature between the aecial stages of the two rusts in Europe. Although *C. ledi* is not known to occur in North America, certain morphological features of *Ledum* rusts from both continents suggest a common ancestor. The peridial cells of *C. ledi* are remarkably similar to those of *C. ledicola* in North America (compare Figs. 2.8, H, and 2.11, I), and the peridium of both species shreds in a similar manner during aeciospore release (Fig. 2.8, A). The western and coastal varieties of *C. ledicola* are also sometimes similar in aeciospore and urediniospore size to *C. ledi* and may possess an indistinct groove (Figs. 2.10, D; 2.11, C, D). Although *C. ledicola* is chiefly epiphyllous on its *Ledum* host, it is occasionally found on the leaf undersurface. The coarse peridial cells of *C. neoglandulosi* and the fine tapered warts (Fig. 2.14) also suggest phylogenetic affinity with *C. ledi*; both aeciospores and urediniospores of *C. neoglandulosi*, however, are considerably smaller (Fig. 2.9).

The occurrence of *C. ledi* and *C. woroninii* on the same host plants has, in the past, led to the conclusion that they are different states of the same fungus (Liro 1907). Field observations and inoculation experiments have confirmed that they are different species that produce different signs and symptoms on both hosts (Tranzschel 1903; Crane et al. 2000*b*).

In northern Europe, rainy summers favor heavy infection of spruce by *C. ledi* (Melekhov 1946; Jalkanen 1993). Trees over large areas may turn bright yellow. Premature shedding of needles probably affects incremental growth.

Specimens examined: On *Ledum palustre*. **FINLAND:** West of Kemijärvi, 7 Aug 1998, P.E.C. (CFB 22203); Kaihuavaara, 7 Aug 1998, P.E.C. (CFB 22205). **GERMANY:** Pommena, Wald vor Stolpmünde, May 1889, P. Sydow (PUR F23); Berlin, Grunewald, 29 Jun 1895, P. Magnus (PUR 577, Type locality). **RUSSIA:** Shisuka, S. Saghalien [Sakhalin], 12 Aug

1928, N. Hiratsuka (PUR 12687). **SWEDEN:** Talum, E Museo Botanico Upsaliensi, 6 Jul 1900, O. Juel (DAVFP 19570).

On *L. palustre* ssp. *diversipilosum* Hara. **JAPAN:** Horomui, Ishikari [Hokkaido], 14 Jun 1925, N. Hiratsuka (PUR F512); 10 Sep 1923 (PUR F 12684); Mt. Nupukaushi, Tokachi [Hokkaido], 6 Jun 1925, N. Hiratsuka (PUR F12685); Mt. Kuro-dake, Ishikari [Hokkaido], 5 Aug 1925, N. Hiratsuka (CFB F12686).

On *Picea abies*. **FINLAND:** Vikingarri, 12 Aug 1995, Y.H. (CFB 22017, 22019); Kittia, 13 Aug 1995, Y.H. & R. Jalkanen (CFB 22061); Ruka, Kuusamo, 20 Sep 1983, R. Jalkanen (CFB 22159); Sierita, Rovaniemi, 24 Aug 1981, R. Jalkanen (CFB 22160); Siperia, Rovaniemi, 15 Sep 1997, R. Jalkanen (CFB 22161) and 28 Aug 1998 (CFB 22178); west of Kemijärvi, 7 Aug 1998, P.E.C. (CFB 22204); Mustiala, Jul 1879, P.A. Karsten (PUR 517, as *Peridermium coruscans*). **RUSSIA:** Levashovo nr. Petersburg, Aug 1882, W. Tranzschel (PUR F316). **SWEDEN:** "Dalaro" nr. Stockholm, Aug 1899, G. v. Lagerheim (PUR F514). **SWITZERLAND:** 31 Jul, 8 Aug. 1891, W. Krieger (PUR F515).

On *P. glauca*. **FINLAND:** Siperia, Rovaniemi, 28 Aug 1998, R. Jalkanen (CFB 22177).

On *P. mariana*. **FINLAND:** Siperia, Rovaniemi, 28 Aug 1998, R. Jalkanen (CFB 22179).

Chrysomyxa ledicola (Arthur) P. Syd. & Syd., Monogr. Ured. III: 507–508. 1915.

Figs. 2.10, A–J; 2.11, A–I

=*Melampsoropsis ledicola* (Peck) Arthur, N. Am. Flora, 7: 119. 1907. [Based on telia]

≡*Uredo ledicola* Peck, Ann. Rep. N.Y. State Mus. 25:90. 1873. [Based on uredinia]

=*Chrysomyxa ledicola* (Peck) Lagerh., Tromsø Mus. Aarsh. 16: 119. 1893. [*nom. nud.*; telia not described]

=*Peridermium decolorans* Peck, Ann. Rep. N.Y. State Mus. 27: 104–105. 1875.

≡*Aecidium decolorans* (Peck) Farl., Bibl. Index I: 38. 1905.

=*Dicaeoma ledi* (Berk. & M.A. Curtis) Kuntze, Rev. Gen. III: 469. 1898.

≡*Puccinia ledi* Berk. & M.A. Curtis, Grevillea, III: 54. 1874.

Type: On *Ledum groenlandicum* (as *L. latifolium*), Mt. Washington, New Hampshire, Jul 1884, E. Faxon. This collection (HUH), with identical collector, location, and date as Ellis & Everhart North American Fungi No.1883 (HUH), cited by Sydow and Sydow (1915), is herein designated as neotype because No. 1883 lacks telia.

Hosts and distribution: The uredinia and telia of *C. ledicola* occur wherever *L. groenlandicum* or *L. decumbens* are found, throughout the boreal forests from Greenland to Alaska; south to northern USA, including Maine, New Hampshire, New York, Wisconsin, and North Carolina in the east, and Washington and Oregon in the west; and in Japan (Hiratsuka et al.1992) and Kamchatka on *L. decumbens* (Ait.) Lodd. (Azbukina 1974). Savile (1969) states that pure *L. glandulosum* is resistant. However, one sample found in Banff National Park appeared to be on pure *L. glandulosum*. *Rhododendron calostrotum* Balf.f. & Kingdon-Ward, *R. griersonianum* Balf.f. & Forrest, *R.* x sp. are also susceptible (Farr et al. 1996; Eglitis et al. 1966).

Spruce species affected are *P. engelmannii*, *P. glauca*, *P. mariana*, *P. pungens*, *P. sitchensis*, and *P. rubens*. European *P. abies* grown in North America as an ornamental is also susceptible. In Japan and Kamchatka, *C. ledicola* is unknown on spruce, although telia form on *Ledum* (Kuprevich and Tranzschel 1957; Azbukina 1974, 1984; Hiratsuka et al. 1992).

Description: On *Ledum* or *Rhododendron*. *Uredinia and telia* epiphyllous on irregular reddish discolored parts of leaves of previous year; uredinia rarely hypophyllous on the leaf midvein, on west coast of North America may occur on flower pedicels and seed capsules. *Uredinia* 0.2–0.6 mm wide, in circular groups or single and scattered, subepidermal, covered by a peridium of one or two layers of cuboid to oblong cells with walls 2 μm thick, dehiscing at apex by rupture of epidermis. *Urediniospores* globose, subglobose, ovoid or ellipsoidal, sometimes with an inconspicuous shallow, longitudinal, wart-containing indentation, 21–44 × 14–37 μm, reddish orange (7A8); wall hyaline, 0.8–1.6 μm thick; *wall + warts* 1.6–4.9 μm thick. *Telia* form irregular confluent crusts, gelatinous when young, erumpent through epidermis, orange (6B7) to reddish brown (

8E8). *Teliospores* catenulate, cuboid to oblong, 18–24 × 12–16 μm. *Basidiospores* globose to slightly pyriform or ovoid, with small apiculus, 7.5–12 × 6.5–11 μm.

On *Picea*. *Spermogonia and aecia* on current-year needles or on cone scales. *Spermogonia* numerous, amphigenous; on *P. sitchensis*, lacking; pale when young, becoming blackened or deep reddish-brown when dry; in cross section, hymenium flat to concave, 100–200 μm wide × 90–150 μm high. *Spermatia* 2–4.1× 1.6–3.3 μm. *Aecia* amphigenous, variable in length along needle, up to 4 mm. *Aeciospores* mainly globose or subglobose, but often ellipsoidal or ovoid, extremely variable in size, depending on location, 23–60 × 18–54 μm, sometimes with an inconspicuous shallow, longitudinal, wart-containing indentation, deep orange or reddish orange (6A8 or 7A8); warts multiannulate, with complex basal connections, giving them a "stellate appearance" in surface view; wall hyaline, 0.8–2.5 μm thick; *wall + warts* 1.6–5.7 μm thick. *Peridium* colorless, not persistent, remaining only as a fringe around the mature aecia; exterior of cells deeply concave, with broad coarsely striate side walls (4–10 μm) and shallow rugulose ornamentation elsewhere; interior of cells convex to slightly concave with dense ornamentation of narrow tapering warts, sometimes arranged in undulating rows, but becoming striated towards the edges.

Notes: *Nomenclature, type, and history of name*

It is curious that for nearly 100 years, Lagerheim (1893, p. 119) has been cited as the author of the name *C. ledicola*. Although he appears to have been the first to place the rust in the genus *Chrysomyxa*, he neither describes nor mentions the telia in this publication, but merely makes a cursory comparison with *C. empetri*, for which he mentions observing telia. Arthur (1912) cited Lagerheim's combination when he placed this epiphyllous species in *Melampsoropsis* (Arthur described the telia). Arthur later abandoned the genus *Melampsoropsis*, but Lagerheim is still cited as the author of *C. ledicola*. Although Saccardo (1888) attributes a description of telia to Berkeley and Curtis (1874) when they described this rust as *Puccinia ledi*, it is not clear from their original description that they saw telia ("spores...springing from thick hyaline pedicels").

Sydow and Sydow (1915) appear to have provided the first valid description of the combination *C. ledicola* and should be cited as the authors of the name.

Regional variation and morphology

Examination of samples of *C. ledicola* from across North America has shown for the first time the extreme variability in certain morphological characters, particularly spore size. It is likely that *C. ledicola* consists of a complex of species or subspecies. However, because there appear to be gradations in certain features, it would be unwise, without further study, to assign these variations new names, especially since the effect of climate and host on such characters as spore size is not known. For comparison, samples were chosen from three geographic areas where regional variations were found: west coast samples associated with *Picea sitchensis*; interior west; and east, including the maritime provinces of eastern Canada and the northeastern USA. These are compared in Table 2.3 and Figs. 2.10 and 2.11. The general trend is for spores to become much larger from the west towards the east, so that some aeciospores in the northeastern USA are almost twice the size of those from the Rocky Mountains. Along with the much larger size in the east, warts are much larger and have more annuli (Figs. 2.10, 2.11), and the ornamentation of peridial cells is much coarser, although they have the same general appearance as those from elsewhere. Both urediniospores and aeciospores from the west coast and most from the far northwest (Yukon and Northwest Territories) have a subtle longitudinal, wart-filled indentation (Figs. 2.10, D; 2.11, C, D); this feature was present in about half of the interior western samples examined, gradually disappearing towards the east. Spore size of the west coast samples and interior western ones is very similar, but the west coast samples on spruce lack spermogonia. Inoculation of spruce species other than *P. sitchensis* with west coast basidiospores from *Ledum* is needed to confirm whether this is a stable character or an effect of the host, particularly since the smaller-spored *Peridermium zilleri* on *P. sitchensis* also lacks spermogonia. The single specimen of *C. ledicola* on *L. palustre* examined from Japan was morphologically consistent with the North American west coast specimens.

Table 2.3. Comparison of *C. ledicola* from three regions of North America[a]

Character	West coast	Interior west	East
Uredinia	Individual, scattered; upper leaf surface, flowers pedicels, capsules, petioles	In circular groups; upper leaf surface, occasionally lower surface on midvein	In circular groups; upper leaf surface
Urediniospores			
Size, μm	25–40 × 16–36 ($\bar{x}$ = 32.0 × 24.8)	23–42 × 14–32 ($\bar{x}$ = 29.4 × 23.5)	28–42 × 24–36 ($\bar{x}$ = 35.9 × 28.5)
"Indentation"	Yes	Yes or no	No
No. wart annuli	3–4	3–4	4–7
Wall, μm	<1	0.8–1.6	1.6
Wall + warts, μm	2.5–3.3	1.6–4.9	4.1–4.5
Aeciospores			
Size, μm	(24)29–50 × 18–36 ($\bar{x}$ = 36.3 × 29.1)	23–51 × 18–34 ($\bar{x}$ = 31.2 × 25.4)	29–60 × 26–54 ($\bar{x}$ = 42.8 × 37.7)
"Indentation"	Yes	Yes or no	No
No. wart annuli	5	3–4	5–7
Wall, μm	0.8–1.6	0.8–1.2	0.8–2.5
Wall + warts, μm	2.5–5.7	1.6–4.9	2.4–4.5
Shape	Variable, but more often ovoid or ellipsoidal than globose	Variable, globose to ellipsoidal or ovoid	Globose or subglobose
Spermogonia	No	Yes	Yes

[a]West coast includes coastal Alaska, British Columbia (including Queen Charlotte Islands), and Washington; Interior west includes central Alberta and the Rocky Mountain region of western Alberta and eastern British Columbia; East includes the the eastern United States (New York, New Hampshire, Wisconsin, North Carolina) and Canada (Nova Scotia, Prince Edward Island, Newfoundland and Gaspé region of Quebec).

Distinguishing features from other rusts

It is clear from the foregoing discussion and Table 2.3 that spore size alone is a very unreliable character in identifying *C. ledicola*, as are such characters as wart size, distance between warts, and wall thickness (Savile 1950, 1955). Moreover, the common name "large-spored spruce – Labrador tea rust" (Ziller 1974) is inappropriate when applied to collections from the west, where the aeciospore size of the "small-spored spruce – Labrador tea rust" (*C. nagodhii*) may actually be similar or even larger than *C. ledicola*. A combination of the following distinctive characters can be used to identify the aecial state of *C. ledicola*: a tendency for spores to be globose rather than elongated; absence of a "cap" or distinct smooth stripe, but spores may have an inconspicuous longitudinal indentation; coarse, multiannulate warts with a "stellate appearance" due to prominent, complex basal connections; evanescent peridium with cells having broad, coarsely striate side-walls. In all fresh samples examined from western Canada the spores were a deep reddish orange color, but this cannot be confirmed for west coast or eastern collections, for which only faded herbarium material was available. Four other species of *Chrysomyxa* occur on *Ledum* spp., *C. ledi*, *C. nagodhii*, *C. reticulata*, and *C. woroninii*, but *C. ledicola* is the only one with epiphyllous uredinia and telia.

Although it has been stated that the aecial state of *C. ledicola* is indistinguishable from *C. empetri* (Savile 1950), a combination of characters, clearly shown by SEM, can be used to distinguish between them (see Table 2.2 and Notes under *C. empetri*).

Life cycle

Fraser (1911, 1912) proved by inoculation of *P. glauca* with basidiospores from *L. groenlandicum* in Nova Scotia that *Peridermium decolorans* is the aecial stage of *C. ledicola*. Successful inoculation of *P. glauca* with the epiphyllous *Ledum* rust during this study confirms the connection for the western interior variety. Although in west coast samples, the morphological similarity of aeciospores on *P. sitchensis* and urediniospores on *Ledum* sp. supports their relationship, experimental confirmation is needed. Observation of *C. ledicola* on *L. groenlandicum* in Alberta over several field seasons

shows that the rust overwinters as mycelium within reddish discolored patches in leaves of the previous season. In early spring, telia form, followed by uredinia, which may develop within or separately from the telia. In midsummer, the old infected leaves fall off, but because the uredinia do not repeat during the same growing season, the *Ledum* plants may appear very healthy for the rest of the summer. In the fall, however, the new infections in the current-year leaves, caused either by urediniospores or aeciospores, become evident by the reddish patches on the leaves. In early spring maturity of telia coincides with opening of vegetative spruce buds, and the young susceptible needles become infected by basidiospores from adjacent *Ledum*. Savile's (1955) observations in the far north, i.e. that uredinia seldom repeat during the growing season in most species of *Chrysomyxa*, suggest that this is likely the common pattern for the life cycle of *C. ledicola* throughout the northern boreal forests. From the west coast, however, one collection had abundant uredinia on flower parts and capsules (PUR 4777, collected Aug 31), suggesting that in this milder climate the life cycle may be somewhat different, perhaps with repeating uredinia. In more northerly, interior regions, flowering of *Ledum* spp. occurs during a brief period in early spring, before or during telia and uredinia formation, and therefore infection is never seen on floral structures. *Chrysomyxa ledicola* is not obligately heteroecious, occurring on *Ledum* spp. far beyond tree line in the north, independent of its spruce hosts (Jørstad 1934; Ziller 1974).

Impact

Chrysomyxa ledicola is the most common and destructive spruce needle rust in North America. Severe outbreaks occur when weather conditions are favorable for infection of spruce needles, and the resulting discoloration is visible in aerial surveys (Conners and Savile 1949; McBeath 1986). Severe infection of up to 90 or 100% of current-year needles has been reported from Alaska (McBeath 1986), Queen Charlotte Islands (Ziller 1974), Manitoba (Conners and Savile 1949), and Alberta (Can. For. Serv. 1971, 1973). Because infected current-year needles are shed at the end of the growing season, the rust may severely affect the next year's growth. In central Alberta, unaffected

trees are often seen adjacent to those with almost 100% of new needles affected. This may result from variability in the timing of bud burst among trees, since infection will occur only if young susceptible needles are present when basidiospores are being produced on adjacent *Ledum* spp. Fraser (1911, 1912) found that buds of *P. rubens* and *P. mariana* opened later than those of *P. glauca* in Nova Scotia, and thus were much less affected by the disease. Because of the potential increment loss due to *C. ledicola*, placement of spruce nurseries and plantations near large populations of *Ledum* spp. is not recommended. The susceptibility of *P. abies*, grown in North America as an ornamental, suggests that this rust could have a negative impact if introduced to Europe and if *L. palustre* should also prove to be susceptible.

Specimens examined: On *Ledum columbianum* (=*L. glandulosum* var. *columbianum*). **USA: Washington:** Long Beach, 1955?, Evans & Brown (PUR 59400).

On *L. glandulosum*. **CANADA: Alberta:** Mi 21, Cascade Fire Rd., Banff Natl. Park, 2 Aug 1963, J. Petty (CFB 5912).

On *L. groenlandicum* ×*glandulosum*. **CANADA: Alberta:** Smith L. Tr., Banff Natl. Park, 21 Jul 1966, V.B. Patterson (CFB 7651).

On *L. groenlandicum*. **CANADA: Alberta:** Red Earth Cr., Banff Natl. Park, 7 Jul 1959, R.T. Ogilvie (CAFB); 3 mi W of Eisenhower Jct., Banff Natl. Park, 16 Jul 1959, R.T. Ogilvie (CAFB); Wadlin L., 9 Jul 1961, A. Machuk, det. L.E. McArthur (CFB 4581); Marmot Basin, Kananaskis For. Exp. Stn., 3 Aug 1969, J.A. Parmelee (CFB 20455, ex DAOM 130357); 31 km N of Hinton, 27 Jun 1995, P.E.C. et al. (CFB 22011); 20 km SE of Grande Cache, 27 Jun 1995, P.E.C. et al. (CFB 22013); Lac la Biche, 19 Jun 1996, P.E.C. (CFB 22023); Smoky L., 5 Jun 1997, P.E.C. (CFB 22068); Red Earth Cr. Tr., Banff Natl. Park, 5 Jul 1997, P.E. & S. Crane (CFB 22124). **British Columbia:** South Westminster, 22 Jun 1922, J.S. Boyce (PUR 4794); Vancouver, 11 Jun 1922, E. Bethel (PUR 65289). **Newfoundland:** St. Anthony's Bight, nr. St. Anthony, 5 Jul 1951, D.B.O.S. & J. Vaillancourt (PUR 54535, ex DAOM 45500); Mealy Mts., Labrador, 11-15 Jul 1950, J.M. Gillett, & W.I. Findlay (PUR 53203, ex DAOM 26537). **Northwest Territories:** Ft. Liard Tower, 12 Jul 1966, E. Gautreau & Y.H. (CFB 7625). **Quebec:** Great Whale R., 12 Jul 1949, D.B.O.S. (PUR 52013, ex DAOM 23505). **USA: Alaska:** Pitchfork Falls, Skagway, Jul 1930, H.E. Parks (PUR 44375). **New York:** Mt. McIntyre, 13 Jul

1923, H.D. House (PUR 4789). **Washington:** Tacoma, 31 Aug 1907, E.W.D. Holway (PUR 4777).

On *L. decumbens*. **CANADA: British Columbia:** Mi 90, Haines Hwy., nr. Tatshenshimi R., 18 Jul 1948, D.V. Baxter (PUR 59399). **Northwest Territories:** 25 mi N & 4 mi E of Ft. Simpson, 15 Jul 1965, E. Gautreau & J. Petty (CFB 6750); Vaillant L., 100 mi S. of Coppermine, 66°13' N, 114°30' W, 27 Jul 1966, E. Gautreau et al. (CFB 7691); Nr. Inuvik, 14 Jul 1981, S. Zoltai (CFB 22193); Inugsuin Fiord, Baffin Is., 69°37' N, 70°02' W, 25 Jul 1967, J.A. Parmelee & J.R. Seaborn (CFB 8711, ex DAOM 117845). **USA: Alaska:** Prince William Sound, 25 Jun 1899, W. Trelease (PUR 4753).

On *L. palustre* var. *procumbens*. **JAPAN:** Mt. Kuro-dake, Prov. Ishikari [Hokkaido], 18 Aug 1925, N. Hiratsuka (PUR F12688).

On *Picea abies*. **USA: Wisconsin:** Jul 1981, E.L. Chambers (PUR 49817).

On *P. engelmannii*. **CANADA: Alberta:** Spray R. Rd., Banff Natl. Park, 6 Aug 1952, R.J. Bourchier (CFB 329). **British Columbia:** Radium Hot Springs, 5 Aug 1945, G. Nasadyk (DAVFP 2842).

On *P. glauca*. **CANADA: Alberta:** Edith Cavell Rd., Jasper Natl. Park, 25 Aug 1952, E.F. Thornton (CFB 320); 85 mi SW of Grande Prairie, 27 Aug 1963, G.J. Smith (CFB 5743); Hargwen Rd., E of Obed Summit, 9 Aug 1996, Y.H. & P.E.C. (CFB 22044); Edmonton, 5 Aug 1996, P.E.C. (CFB 22043, from inoculation); 34.5 km E of Obed Summit, Hwy. 16, 9 Aug 1996, P.E.C. (CFB 22047); Smoky L., 14 Aug 1996, P.E.C. (CFB 22051, 22052), 26 Sep 1996 (CFB 22067); approx. 10 km S of Cynthia-Lodgepole turnoff on Hwy. 16, Aug 1997, P.V. Blenis (CFB 22139). **Newfoundland:** St. Anthony, 30 Jul 1951, D.B.O.S. & J. Vaillancourt (PUR 54536, ex DAOM 45496). **Northwest Territories:** Ft. Resolution, 23 Aug 1963, A. Machuk & J. Petty (CFB 5916). **Nova Scotia:** Pictou, 24 Jul 1910, W.P. Fraser (PUR 4712, from inoculation). **Quebec:** Great Whale R., 7 Aug 1949, D.B.O.S. (CFB 8764, ex DAOM 23437); Seven Islands, Saguenay Co., 2 Aug 1907, C.B. Robinson (PUR 4706); Gaspé Peninsula, Jul 1921, B.T. Dickson (PUR 4716). **Saskatchewan:** Prince Albert Natl. Park, 20 Aug 1996, C. Zelmer (CFB 22065). **Yukon Territory:** Midnight Dome, Dawson City, 12 Jul 1972, J. Susut (CFB 21860); Dawson, 20 Aug 1962, J. Holms (DAVFP 14562). **USA: New Hampshire:** Second Conn. L., Pittsburg, 17 Aug 1933, Spaulding et al. (PUR 59403) **North Carolina:** Ashville, Jul 1926, A.H. McAndrews (PUR 59394).

On *P. mariana*. **CANADA: Manitoba:** Lynn L., 22 Aug 1967, C. Tidsbury (WINF(M) 8026); Lynn L., 20 Aug 1969, W. Crawford (WINF(M) 12166). **Saskatchewan:** Candle L., 20 Aug 1952, R. Whitney (WINF(M) 1948). **USA: New York:** Mt. Colvin, Adirondack Mts., Aug 1873, C.H. Peck (PUR 4736, Type of *Peridermium decolorans*).

On *P. rubens*. **CANADA: Prince Edward Island:** 8 Sep 1888, Macoun? (PUR 4744).

On *P. sitchensis*. **CANADA: British Columbia:** Tlell, Queen Charlotte Is., 31 Jul 1946, J.B. Scott (CFB 22098, ex DAVFP 2571); Port Clements, Queen Charlotte Is., 27 Aug 1943 (CFB 22099, ex DAVFP 328); Prince Rupert, 14 Aug 1951, R.E. Foster (CFB 22100, ex DAVFP 7018); Terrace, 20 Jul 1961, A.K.D. Jardine (CFB 22104, ex DAVFP). **USA: Alaska:** Sitka, 5 Aug 1914, J.P. Anderson (PUR 43499). **Washington:** Bainbridge Is., Kitsap Co., 29 Jul 1909, E. Bartholomew (PUR 4747); Long Beach, 3 Aug 1955, Wheeler et al. (PUR 54538).

C. monesis Ziller, Can. J. Bot. 32: 435–437. 1954. **Figs. 2.12, A–I**

Type: On *Moneses uniflora*, Queen Charlotte City, Queen Charlotte Islands, British Columbia, 10 Jul 1952, W.G. Ziller (Holotype DAVFP 7789! Isotype PUR 53312!).

Hosts and distribution: *Picea sitchensis* is the only known aecial host of *C. monesis* on the Queen Charlotte Is. and coastal B.C. Uredinia and telia are found only on *Moneses uniflora* (L.) Gray and *M. uniflora* var. *reticulata* (L.) Gray on the west coast of North America from Alaska to Washington, and in a few locations further inland in British Columbia (Savile 1955; Ziller 1974). It can survive on this host independent of host alternation (Ziller 1974).

Description: On *Moneses*. Systemic in leaves, causing slight atrophy and yellowing of foliage. *Uredinia* hypophyllous and on petioles, stems, peduncles and flowers, 0.4–0.8 mm in diam, subepidermal, pulvinate, light orange yellow; peridium not seen. *Urediniospores* ovoid or ellipsoidal, occasionally subglobose, 22–34 × 14–22 μm, catenulate with intercalary cells; wall indistinct, *wall + warts* 1.6–4.1 μm thick. *Telia* hypophyllous on leaves of previous year, orange, waxy, ~0.2–0.3 mm in diam. *Teliospores* 12–22 × 6–12 μm; wall 1 μm thick (Ziller 1974).

On *Picea*. Systemic in cones, causing premature opening. *Spermogonia and aecia* on cone scales. *Spermogonia* forming irregular continuous layers 500–700 μm wide. *Aecia* at base of scale between bract and scale, irregular, confluent, about 3–4 × 4–6 mm in diam, bullate. *Aeciospores* globose, subglobose, to ellipsoidal, 28–45 × 17–32 μm; wall indistinct, warts in surface view irregular or elongated, not crowded; in side view narrow, annulate, fluted at bottom, with basal connections; *wall + warts* 4.5–6.6 μm thick. *Peridium* evanescent, cells globose to ovoid, rugose, warts separate or forming irregular ridges.

Notes: Ziller (1954) proved the connection of the telial and aecial states of *C. monesis* with inoculation experiments of both *Moneses uniflora* and *Pyrola* spp. Aeciospores from infected cones of *Picea sitchensis* produced uredinia and telia only on *M. uniflora*. Although similar in symptoms and life cycle to *C. pirolata*, this rust has a much more restricted distribution and host range. It is significant that *Pyrola* spp. are rare on the Queen Charlotte Islands (Calder and Taylor 1968) and that the distribution of the rust coincides with the geographical distribution of *P. sitchensis*, the only known aecial host (Ziller 1954). Isolation and specialization to these particular hosts may have resulted in the distinct morphology. The warts on both urediniospores and aeciospores of *C. monesis* are narrow and somewhat fluted (Fig. 2.12, D–G), whereas those of *C. pirolata* are broad, pulvinate, polygonal or elongated.

Infection of sitka spruce cones by *C. monesis* prevents seed formation, and may be economically important where cones are collected for reforestation or export (Ziller 1954).

Specimens examined: On *Moneses uniflora*. **CANADA: British Columbia:** 5 mi from Massett, Queen Charlotte Is., 1 Sep 1956, W.G.Z. (CFB 8605, ex DAVFP 10111); Massett, Q.C.I., 7 Jul 1952, W.G.Z. (PUR 53312, Holotype). **USA: Alaska:** Sitka, 10 May 1914, J.P. Anderson (PUR 4619).

On *Picea sitchensis*. **CANADA: British Columbia:** North shore, Graham Is., Queen Charlotte Is., 1 Sep 1956, W.G.Z. (CFB 8622, ex DAVFP 10017).

Chrysomyxa nagodhii P.E. Crane, sp. nov. **Fig. 2.13, A–K**

=*Chrysomyxa ledi* de Bary var. *groenlandici* Savile, Can. J. Bot. 33: 490. 1955. [*nom. nud.*; telia not described]

=*Melampsoropsis abietina* Arthur, N. Am. Flora, 7: 119. 1907. *p.p.*

Type: On *Ledum groenlandicum* Oeder, near Obed Summit, Hwy. 16, E of Range Rd. 221, AB, P.E. Crane (Holotype DAOM, Isotypes DAVFP, PUR, CFB 22207).

Hosts and distribution: This species probably occurs throughout the range of *Ledum decumbens* and *L. groenlandicum* in North America, independent of host alternation. This includes Nova Scotia to British Columbia, the Yukon and Northwest Territories, and Alaska, south to Connecticut, Michigan, New Hampshire, New York, and Wisconsin. One collection on *Rhododendron* sp. from Whalley, B.C., reported as *C. ledi* var. *rhododendri*, was re-identified as *C. nagodhii*. The aecial state has been verified on *P. engelmannii*, *P. glauca*, *P. mariana*, *P. pungens*, and *P. rubens*. Collections identified as *C. ledi* on *P. sitchensis* have so far proven to be inconsistent with *C. nagodhii* (see Notes under *C. vaccinii* and *Peridermium zilleri*). The identity of many previous North American records (as *C. ledi* or *C. ledi* var. *groenlandici*) on spruce is in doubt because of confusion with species of similar spore size, including *C. cassandrae*, *C. chiogenis*, and the small-spored western North American form of *C. ledicola*.

Description: In *Ledo* vel *Rhododendro*. *Uredinia et telia* hypophyllosa in foliis anni superioris, maculas flavas et aurantiacas efficientia. *Uredinia* 1/4 mm lata, cupulata, cum peridio incohato. *Urediniosporae* ellipsoideae, ovoideae, subglobosae, vel irregulares, truncatae in extremo altero vel utroque, 15–36 × 12–22 μm, meloni-flavae; tunica levis prope extrema sporae, sed in lateribus cum verrucis humilibus, dense congregatis, indistinctis, in summo planis et magnitudine et forma variis; *tunica et verrucae* 0.8–2.5 μm crassae. *Telia* valde aurantiaca, discreta, gelatinose, 0.1–0.2 mm lata. *Teliosporae* cuboideae, ellipticae quando maturant, lateriores ad summum sori, 12–23 × 15–28 μm. *Basidiosporae* globosae ad subglobosae cum apiculo parvo, et magnitudine et forma irregulares, 6–13 × 6–12 μm.

In *Picea*. *Spermogonia et aecia* in lineis distinctis et robigo-flavis in acuibus arboreis huius anni. *Spermogonia* amphigenosa, prominens, robigo-brunnea quando exsiccata, per mediam partem magis vel minus globosa, hymenium concavum ad aliquantum planum, 70–200 μm latum x 60–163 μm altum. *Spermatia* ellipsoidea, aliquando globosa, 3–4.5 × 2–3.5 μm. *Aecia* amphigenosa, 1/4–2 mm lata, tubularia, separata. *Aeciosporae* forma et magnitudine variae, globosae, subglobosae, ellipsoideae vel ovoideae, cum tegimento humili in extremo altero vel utroque, pars lineae longitudinalis et leviter-marginatae per latus alterum sporae, 15–31 (–34) ×14–24 μm, valde aurantiacae, tunica hyalina, <0.8 μm crassa; verrucae congregatae, fastigatae, latitudine variae, annulatae, superficies tegamenti prope levis; *tunica et verrucae* 1.6–4.1 μm crassae. *Peridium* scindet in maturitate, cellulae facile separant; superficies externa cellulae alte concava, cum marginibus distinctis, tunica rugulosa; superficies interna cellulae paulo concava cum margine elevata, verrucae humiles, irregulares, discretae et leves vel dorsa humilia formantes, aliquando reticulatae videntur.

Etymology: Named for *nagodhi*, the aboriginal (Chipewyan) name for the major telial host, *Ledum groenlandicum*.

On *Ledum* or *Rhododendron*. *Uredinia and telia* hypophyllous on leaves of previous year, where it produces a yellow and orange mottling. *Uredinia* 1/4 mm wide, cupulate, with a rudimentary peridium. *Urediniospores* ellipsoidal, ovoid, subglobose, or irregular, with one or both ends truncate, 15–36 × 12–22 μm, melon-yellow (5A6); wall smooth at ends of spore, but with shallow, closely crowded, indistinct, flat-topped warts of variable size and shape on sides; *wall + warts* 0.8–2.5 μm thick. *Telia* deep orange (5A8 or 6A7), discrete, gelatinous, 0.1–0.2 mm wide; seen in cross section, hymenium is flat. *Teliospores* cuboid, becoming elliptical as they mature, wider toward top of sorus, 12–23 × 15–28 μm. *Basidiospores* globose to subglobose with a small apiculus, irregular in size and shape, 6–13 × 6–12 μm.

On *Picea*. *Spermogonia and aecia* on distinct rusty-yellow bands on current-year needles. *Spermogonia* amphigenous, prominent, rusty-brown when dry; in cross section ± globose, hymenium concave to somewhat flattened, 70–200 μm wide × 60–163 μm

high. *Spermatia* ellipsoidal, occasionally globose, 3–4.5 × 2–3.5 μm. *Aecia* amphigenous, 1/4–2 mm wide, tubular, separate. *Aeciospores* variable in shape and size, globose, subglobose, ellipsoidal or ovoid, with a shallow cap at one or both ends, part of a smooth-edged longitudinal cap along one side of spore, 15–31(–34) × 14–24 μm, deep orange (5A8); wall hyaline, <0.8 μm thick; warts crowded, tapered, variable in width, annulate, surface of cap nearly smooth; *wall + warts* 1.6–4.1 μm thick. *Peridium* shreds at maturity, cells separate easily; outer cell surface deeply concave, with sharply defined edges, wall rugulose; inner cell surface shallowly concave with a raised edge, warts shallow, irregular, discrete and fine or forming shallow ridges, sometimes appearing reticulate.

Notes: Although this North American hypophyllous *Ledum* – spruce rust has been considered conspecific with the European *C. ledi*, the different *Ledum* host species and distinct morphology, especially of the aeciospores and urediniospores, provide strong evidence that it should be considered a separate species. Savile (1950, 1955) observed slight morphological differences between the European and North American forms and placed *C. nagodhii* as a variety of *C. ledi*, but because he did not describe telia, the variety name is considered invalid. The almost-smooth appearance of the urediniospores (Fig. 2.13, D, E) of *C. nagodhii* is unique within the genus and very different from the evenly warted spores with a narrow groove seen in *C. ledi* (Fig. 2.8, D). Similarly, the smooth caplike stripe of *C. nagodhii* aeciospores (Fig. 2.13, G) is distinct from the well-defined narrow warted groove seen in *C. ledi* aeciospores (Fig. 2.8, G). *Chrysomyxa nagodhii* is an exception to the "rule" that in *Chrysomyxa* the morphology of the aeciospores and urediniospores of a given species is almost identical (Savile 1950).

The telia of *C. nagodhii* appear in the early spring, followed by the uredinia, which may be present throughout the growing season. The yellow-orange leaf mottling produced by this rust on *L. groenlandicum* differs from the deep reddish discoloration induced by *C. ledicola*. This rust fungus often occurs on the same spruce trees, and even the same needles, as *C. ledicola*, but has a more sporadic occurrence than the latter. In such cases, even under a dissecting microscope, the smaller spore size, paler orange color,

and more persistent peridium of *C. nagodhii* are helpful in distinguishing the two species. Distinguishing *C. nagodhii* from *C. cassandrae* in the aecial stage is much more difficult, and the broadleaved hosts, *Ledum* and *Chamaedaphne*, occupy very similar boggy habitats. The subtle differences in their aecial characters are listed in Table 2.1.

The connection between the telial stage on *L. groenlandicum* and the aecial stage on spruce was proven experimentally by Fraser (1911, 1912) and Arthur (1912), and confirmed during this study. Fraser's inoculated material on *P. rubens* and from this study on *P. mariana* and *P. pungens* were compared and used to formulate the species description.

The distinct morphology of the urediniospore surface of *C. nagodhii* suggests a unique dispersal mechanism of these spores. The spore surface is sticky and the spores are extruded in long columns that remain intact if undisturbed (Fig. 2.13, A). Brown-bodied oribatid mites, frequently observed among the uredinia on freshly collected *L. groenlandicum* leaves in central Alberta, carry urediniospores of *C. nagodhii* on their body surface (Fig. 2.13, K). These mites have recently also been found on *L. groenlandicum* in Ontario and Wisconsin and have been described as a new species, *Dentizetes ledensis* V.M. Behan-Pelletier (Acari: Oribatida: Ceratozetidae) (Behan-Pelletier 2000). They were also present in a collection of *C. nagodhii* from Newfoundland, suggesting that they occur throughout the range of *L. groenlandicum* in North America. It is possible that they play a role in the dispersal of this fungus on the broadleaved host.

Planting of cultivated rhododendrons close to areas with infected *L. groenlandicum* is not recommended because of the ability of this rust to infect these closely related host plants.

Specimens examined: On *Ledum decumbens*. **CANADA: British Columbia:** Mi 402, Alaska Hwy., Summit Lk., 15 Jun 1972, C.S. Wood (CFB 22095, ex DAVFP 20264).

On *L. groenlandicum*. **CANADA: Alberta:** 3 mi W, 0.5 mi S of Harman Valley, 56°06' N 116°55' W, 15 Jun 1965, C.R. Layton (CFB 6672); 2 mi W of Miette Hot Springs, Jasper Natl. Park, 53° 07' N 117° 48' W, 5 Aug 1966, P.J. Maruyama & Y.H. (CFB 7516); Sir Winston

Churchill Prov. Park, Lac la Biche, 19 Jun 1996, P.E.C. (CFB 22023); Hargwen Rd., E of Obed Summit, Hwy. 16, 26 Jun 1996, Y.H. et al. (CFB 22035); Beaver Hills L. Tr., Jasper Natl. Park, 6 Sep 1996, P.E.C. (CFB 22064); Pine Ridge Nursery, Smoky Lake, 26 Sep 1996, P.E.C. (CFB 22066); Red Earth Cr., Banff Natl. Park, 10 Jul 1998, P.E.C. (CFB 22189). **British Columbia:** Prince George, 16 Sep 1955, W.G.Z. (DAVFP 9745); Field, 30 Jun 1958, W.G.Z. (DAVFP 10850); South Westminster, 22 Jun 1922, J.S. Boyce (PUR 4794). **Newfoundland:** St. Anthony, 21 Jul 1951, D.B.O.S. & J. Vaillancourt (PUR 54531, DAOM 45442). **Northwest Territories:** 60 mi S of Wrigley, 62° 20' N 123° 30' W, 15 Jul 1965, E. Gautreau (CFB 6698); Ft. Liard Tower, 13 Jul 1966, E. Gautreau & Y.H. (CFB 7625). **Nova Scotia:** Pictou, 15 Jun 1910, W.P. Fraser (PUR 4875). **Quebec:** Great Whale R., 12 Jul 1949, D.B.O.S. (PUR 52013, ex DAOM 23505). **USA: Alaska:** Chicken, 15 Jul 1959, W.G.Z. (DAVFP 11556); Pitchfork Falls, Skagway, Jul 1930, H.E. Parks (PUR 44375). **Wisconsin:** Sturgeon Bay, 24 Jun 1913, J.J. Davis (PUR 4874).

On *Ledum* sp. **CANADA: Manitoba:** Chisel Lk., 18 Aug 1970, R.C. Tidsbury (CFB 20195). **Saskatchewan:** Pasquia Hills, 28 Jul 1970, R.C. Tidsbury (CFB 20163).

On *Rhododendron* sp.: **CANADA: British Columbia:** Whalley, 3 Sep 1958, E. Fridell & C. Gibson (DAVFP 10779, as *C. ledi* var. *rhododendri*).

On *Picea glauca*. **CANADA: Alberta:** Pine Ridge Nursery, Smoky Lake, 14 Aug 1996, P.E.C. (CFB 22053). **British Columbia:** Prince George, 2 Aug 1954, J.A. Calder et al. (DAOM 45454).

On *P. mariana*. **CANADA: Alberta:** Edmonton, 28 Jul 1997 & 4 Aug 1997, P.E.C. (CFB 22133, from inoculation); Edmonton, 25 Jul 1997, P.E.C. (CFB 22134, from inoculation). **British Columbia:** Prince George, 4 Sep 1950, W.G.Z. (DAVFP 6055).

On *P. pungens*. **CANADA: Alberta:** St. Albert, 13 Aug 1997, P.J. Maruyama (CFB 22155); Edmonton, 11 Sep 1996, P.E.C. (CFB 22058); Edmonton, 28 May 1998, P.E.C. (CFB 22186, from inoculation).

On *P. rubens*. **CANADA: Nova Scotia:** Pictou, 31 Jul 1909, W.P. Fraser (PUR 4852, from inoculation); Pictou, 24 Jul 1910, W.P. Fraser (PUR 4854, from inoculation).

Chrysomyxa neoglandulosi P.E. Crane, sp. nov. **Fig. 2.14, A–J**

=*Chrysomyxa ledi* de Bary var. *glandulosi* Savile, Can. J. Bot. 33: 489. 1955. [*nom. nud.*; telia not described]

=*Melampsoropsis abietina* Arthur, N. Am. Flora, 7: 119–120. 1907. *p.p.*

Type: On *Ledum glandulosum* Nutt., Waterton Lakes Natl. Park, start of trail to Little Akamina L., near Cameron L., Alberta, 11 Jul 1998, P.E. Crane (Holotype DAOM, Isotypes PUR, DAVFP, CFB 22174).

Hosts and distribution: *Chrysomyxa neoglandulosi* likely occurs wherever its telial host, *Ledum glandulosum*, is found. This upright shrub is found in moist to wet meadows and forests in the montane to subalpine zones of southern British Columbia east of the Coast–Cascade Mountains, to southwestern Alberta, southward to Montana, Idaho, Wyoming, and northern California (Douglas et al. 1999). The only reported aecial host, *Picea engelmannii*, occurs in similar mountainous habitats in western Canada and USA. According to Gould (1966), *C. neoglandulosi* can also attack rhododendrons.

Description: In *Ledo. Uredinia et telia* hypophyllosa in locis chloroticis in foliis anni superioris, vel caulicolosa. *Uredinia* <1/4 mm diam, sparsa vel in circulis, conicalia dum non aperta, per epidermim erumpentia, cum peridio inconspecto de cellulis irregularibus et tenuiter tunicatis. *Urediniosporae* globosae vel subglobosae, nonnumquam ellipsoideae, 14–28 × 11–23 μm, aurantiacae, tunica hyalina, distincte formata, 0.8–1.6 μm crassae; verrucae magnitudini similes, levissimae et angustae, ad spiculum fastigatae, annulationes inconspectae, coniunctiones basales inter verrucas absunt vel parvum evolutae; tunica cum verrucis 1.6–3.3 μm crassae. *Telia* rotunda vel elliptica, ampliora et magis irregularia quam uredinia, 1/4 - 3/4 mm longa, pruno-armeniaco-flava. *Teliosporae* 12–15 × 18–22 μm. *Basidiosporae* ovoideae, subglobosae, globosae, vel irregulariter formatae, cum apiculo lato, 6–10 × 6–9 μm.

In *Picea. Spermogonia et aecia* in acuibus arboreis huius anni. *Spermogonia* amphigenosa, elliptica, pallide-brunnea; per mediam partem, 90–170 μm alta × 114–144 μm lata, hymenium planum vel tenuiter concavum, cum margine tenui et fusca et distincte formata. *Spermatia* ellipsoidea vel ovoidea, 2.5–3.7 ×1.6–2.5 μm. *Aecia*

amphigenosa, discreta. *Aeciosporae* globosae ad subglobosae, ellipsoideae vel ovoideae, 18–25 × 14–22 μm, fusco-aurantiacae; tunica hyalina, 1.6–2.5 μm crassa, verrucae parvissimae, fere echinulatae, congregatae, annulationes inconspectae; *tunica cum verrucis* 1.6–3.0 μm crassa. *Peridium* simile tubo, persistens; superficies externa cellularum concava, dense rugosa, cum marginibus late et crasse striatus; superficies interna plana ad exigue concava, cum ornamentis paulis et dorso similibus.

Etymology: Named for the telial host plant, *Ledum glandulosum* (*Rhododendron neoglandulosum* Harmaja).

On *Ledum*. *Uredinia and telia* hypophyllous on chlorotic spots on leaves of previous year, or caulicolous. *Uredinia* <1/4 mm in diameter, scattered or in circular groups, conical when unopened, erumpent through epidermis, with an inconspicuous peridium of irregular thin-walled cells. *Urediniospores* globose or subglobose, occasionally ellipsoidal, 14–28 × 11–23 μm, orange (6B8 or 6A8); wall hyaline, well-defined, 0.8–1.6 μm thick; warts even in size, very fine and narrow, tapering to a point, annuli very faint and only visible by SEM, basal connections between warts absent or poorly developed; *wall + warts* 1.6–3.3 μm thick. *Telia* round or elliptical, larger and more irregular than uredinia, 1/4–3/4 mm long, apricot-yellow (5B6). *Teliospores* 12–15 × 18–22 μm. *Basidiospores* ovoid, subglobosė, globose, or irregular in shape, with a broad apiculus, 6–10 × 6–9 μm.

On *Picea*. *Spermogonia and aecia* on current-year needles. *Spermogonia* amphigenous, elliptical, pale brown; in cross section, 90–170 μm high × 114–144 μm wide, hymenium flat or slightly concave, with a well-defined, thin dark margin. *Spermatia* ellipsoidal or ovoid, 2.5–3.7 × 1.6–2.5 μm. *Aecia* amphigenous, discrete. *Aeciospores* globose to subglobose, ellipsoidal or ovoid, 18–25 × 14–22 μm, dark orange (5A8); wall hyaline, 1.6–2.5 μm thick, warts very tiny, almost echinulate, crowded, annuli difficult to discern; *wall + warts* 1.6–3.0 μm thick. *Peridium* tubelike, persistent; outer surface of cells concave, densely rugose, with wide coarsely striate margins; inner surface flat to slightly concave, with shallow ridgelike ornamentation.

Notes: *Chrysomyxa neoglandulosi* was first distinguished from *C. ledi* and from other North American hypophyllous rusts on *Ledum* spp. by Savile (1950, 1955), who designated it as a variety of the European rust. However, because he did not describe the telia, the variety name is invalid and this rust has continued to be called *C. ledi*. The smaller, more globose spores, the different broadleaved host species, and much finer ornamentation of both the urediniospores and aeciospores (Fig. 2.14, C–H) clearly distinguish it from other rusts of the *C. ledi* complex and support separate species status. Two young Engelmann spruce were inoculated in 1998 with basidiospores from *L. glandulosum* collected at Waterton, Alberta. After 10 days, two needles formed spermogonia, but they failed to develop aecia. Two other spruce collected very close (almost touching) infected *L. glandulosum* were brought to Edmonton to observe development of any infection already present; other potential sources of infection, such as *L. groenlandicum*, could not be found in the vicinity of the collected spruce. These trees developed both spermogonia and aecia that were consistent with other purported samples of *C. neoglandulosi*. The similarity of the aeciospores to the urediniospores in size, shape, and surface ornamentation (Fig. 2.14, C–H) leaves little doubt that this is the aecial state of *C. neoglandulosi*.

Chrysomyxa neoglandulosi probably survives in the uredinial state on *L. glandulosum* in the absence of *P. engelmannii*, and telia may not form in certain locations (Krebill 1968). Uredinia are found throughout the growing season on leaves of the previous year, which are later shed. Current-year leaves become infected, but rarely show signs of infection until the next spring, when uredinia, or telia followed by uredinia, form from overwintered mycelium within the leaves (Krebill 1968). In late spring, basidiospores produced by germinating teliospores on *L. glandulosum* infect current-year needles of *P. engelmannii*.

Chrysomyxa neoglandulosi and *C. ledicola* are the only two species of *Chrysomyxa* in North America that do not have aeciospores or urediniospores consistently marked by a longitudinal groove or cap. In both species, the spores are more often globose than elongate, and they have aecial peridial cells with broad, coarsely

striate side walls. However, these species are unlikely to be confused on spruce because *C. neoglandulosi* has much smaller aeciospores than does *C. ledicola*. The extremely fine surface ornamentation of both urediniospores and aeciospores of *C. neoglandulosi* is also unique. Whereas most other North American species have warts with annuli visible by light microscopy, the processes on the walls of *C. neoglandulosi* are finely tapered and appear almost echinulate (Fig. 2.14, E, H). Under SEM, however, the indistinct annuli can be seen on the aeciospores (Fig. 2.14, H). This species is also distinct in having peridial cells with a densely ornamented outer surface (Fig. 2.14, I), rather than the nearly smooth surface characteristic of most of the species studied.

Specimens examined: On *Ledum glandulosum*. **CANADA: Alberta:** Lower Cascade Fire Rd., Banff Natl. Park, 9 Jun 77, P. Achuff (CAFB 810491); 7 mi W of Waterton Lakes Natl. Park, 19 Aug 1966, N.W. Wilkinson (CFB 7464); 2.5 km along Akamina Pass Trail, Waterton Lakes Natl. Park, 6 Jul 1997, P.E.C. (CFB 22174, Isotype; CFB 22079) . **British Columbia:** Arthur L., Falkland, 21 Jul 1952, W.G.Z. (DAVFP 8117); Falkland, 25 Jul 1952, W.G.Z. (DAVFP 8138); Kelowna, 13 Aug 1954, L.M. Wallington & B.A. Sugden (DAVFP 9427); Penticton, 17 Aug 1971, W.G.Z. (DAVFP 19993); Monashee Pass, 5 Jul 1953, J.A. Calder (10109) & D.B.O.S. (PUR 54527, ex DAOM 45484). **USA: Idaho:** Josephus Lakes, Custer Co., 5 Aug 1916, J.F. Macbride & E.B. Payson (PUR 4857). **Montana:** Sylvan Geysers, Yellowstone Natl. Park, 26 Jul 1899, J.B. Ellis (PUR 4856); Alpine, 5 Aug 1921, E. Bartholomew (PUR 4858). **Nevada:** Jarbidge, 7 Jul 1912, Nelson & Macbride (PUR 4859)

On *L. glandulosum* × *L. groenlandicum*. **CANADA: Alberta:** Red Earth Cr., 7 mi from highway, Banff Natl. Park, 7 Jul 1959, R.T. Ogilvie (CAFB); Red Earth Fire Rd., mi 33, Banff Natl. Park, 16 Jun 1965, V.B. Patterson (CFB 7399).

On *Picea engelmannii*. **CANADA: Alberta:** Trail to Akamina L., nr. Cameron L., Waterton Lakes Natl. Park, 10 Aug 1998, P.E.C. (CFB 22184). **British Columbia:** Kelowna, 13 Aug 1954, L.M. Wallington & B.A. Sugden (DAVFP 9390); 1 mi E of Park Headquarters, Manning Prov. Park, 10 Aug 1953, J.A. Calder & D.B.O.S. (PUR 59392). **USA: Idaho:** Scolfield Cr., Clearwater Co., 20 Jul 1965, J.W. Kimmey (PUR 61362).

Chrysomyxa piperiana Sacc. & Trotter ex Cumm., Mycologia, 48: 602. 1956.

Figs. 2.15, A–F; 2.16, A–F

=*Chrysomyxa piperiana* Sacc. & Trotter ex Faull, J. Arnold Arbor. 17: 110. 1936. [Based on telia, but no Latin diagnosis]

=*Melampsoropsis piperiana* Arthur, N. Am. Flora, 7: 120. 1907. [Based on uredinia]

≡*Caeoma piperianum* (Arthur) Sacc. & Trotter, in Saccardo in Sylloge Fung. 21: 787. 1912. [Based on uredinia]

≡*Chrysomyxa piperiana* (Arth.) Sacc. & Trotter, in Sacc. Sylloge Fung. 21: 716. 1912. [Based on uredinia]

=*Peridermium parksianum* Faull, J. Arnold Arbor. 15: 87. 1934.

Type: On *Rhododendron macrophyllum* Don (*R. californicum* Hook.), Trinidad, California, June 1935, H.E. Parks 5565 (Holotype PUR 44390! Isotype K).

Hosts and distribution: The aecial stage of *Chrysomyxa piperiana* is found on *Picea sitchensis* in the Pacific Northwest of North America, including Oregon, California, and Washington. Uredinia and telia occur on native broadleaved California rhododendron (*R. macrophyllum* (=*R. californicum*)) and its hybrids (Ziller 1974). It has been found in the uredinial state in Manning Provincial Park, B.C., at the northern limit of *R. macrophyllum*. Although both uredinia and telia have been recorded on cultivated and wild rhododendron from the west and north coasts of Vancouver Is., the aecial state has not been recorded in Canada (Ziller 1974), perhaps because this rust is adapted to a more Mediterranean climate, with the aecia forming unusually late in the season (September to December) (Savile 1950, 1955). A recent report (Cao et al. 1996) of the aecial state, *Peridermium parksianum*, on *Picea wilsonii* Mast. in northwest China merits further study. Although the description is consistent with *C. piperiana*, the telial stage was not found to confirm the identification.

Description: On *Rhododendron*. *Uredinia and telia* hypophyllous on previous year's foliage, causing yellow to brown leaf spots. *Uredinia* circular, 0.2–1 mm in diameter, with a distinct peridium of large thin-walled cells, 10–14 μm thick (Faull 1936). *Urediniospores* variable in size and shape, ellipsoidal, fusiform, cylindrical,

clavate, or irregular, occasionally with tail-like ends, 30–78 × 15–22 μm, with smoother longitudinal cap on one side; wall hyaline, 0.4–1.2 μm thick; *wall + warts* 2.5–3.7 μm thick. *Telia* waxy, pulvinate, 0.3–1 mm in diam. *Teliospores* subglobose to oblong, 16–35 × 9–19 μm. *Basidiospores* globose to subglobose, pointed at one side, 5.7–12 × 5.3–10 μm.

On *Picea*. *Spermogonia and aecia* on current-year needles. *Spermogonia* epiphyllous, prominent, round to oval, dark brown, arising along stomatal lines; in cross section, hymenium flat to slightly concave and poorly defined, 122–220 μm wide × 116–175 μm high. *Spermatia* globose to ellipsoidal, 3.3–4.1 × 2.5–3.3 μm. *Aecia* on yellowish discolored portions of affected needles, epiphyllous, tubular, 0.5–1 mm wide. *Aeciospores* fusiform, falcate, lanceolate or narrowly ellipsoidal, with a narrow longitudinal smoother area or groove, appearing as a delicate cap at spore ends, 54–114 × 12–18 μm; wall hyaline, <0.8 μm thick; *wall + warts* 2.9–3.3 μm thick. *Peridium* rupturing at the apex, persistent, with brown cells at tip; cells elongated, overlapping, smooth and shallowly concave on outer surface, flat and with fine shallow warts on inner surface.

Notes: Arthur's (1907) original description of this rust as *Melampsoropsis piperiana* and his later combination of *C. piperiana* (1912) were based on uredinia. Faull (1936) described the telia, but not in Latin. Cummins (1956) validated the name by providing the necessary Latin description of the telia. Inoculations to prove the connection of *Peridermium parksianum* and *C. piperiana* were made by Faull (1936).

The extremely long, narrow aecio- and urediniospores of *C. piperiana* are distinctive in the genus. The surface morphology of the spores and the peridial cells is most like *C. roanensis* (see comments under that species).

Spermogonia form in late spring or early summer on spruce needles. Aecia develop slowly and mature in autumn, as late as November or December (Faull 1934).

Specimens examined: On *Picea sitchensis*. **USA: California:** Spruce Cove, Trinidad, 15 Oct 1932, H.E. Parks 4052 (DAVFP 14005, PUR 48213); Eureka, 23 Feb 1997, B. Callan (DAVFP 25254).

On *Rhododendron macrophyllum* (*R. californicum*). **CANADA: British Columbia:** 20 mi from Hope to Princeton, 29 Jun 1950, W.G.Z. (DAVFP 6060). **USA: California:** Trinidad, Jun 1935, H.E. Parks 5565 (PUR 44390, Type). **Oregon:** Newport, 16 May 1914, G.H. Godfrey (PUR 64987); McCredie Hot Springs, above Oakridge, Cascade Natl. For., Lane Co., 10 Jun 1920 (PUR 4891). **Washington:** Seattle, May 1892, C.V. Piper (PUR 4890).

C. pirolata Wint., in Rabenhorst's Kryptogamen-Flora, 1: 250. 1881.

Figs. 2.17, A–H; 2.18, A–H

=*Chrysomyxa pyrolae* (Strauss) Rostr., Mycol. Not. in Bot. Centralbl. 5: 126–127. 1881. [Telia mentioned]

≡*Uredo pyrolae* Strauss [*fide* Rostrup 1881]

=*Chrysomyxa pyrolae* (DC.) Rostr. var. *pyrolata* (Schwein.) Jørst., K. Nor. Vidensk. Selsk. Skr. 38: 51–52. 1935. [Based on uredinia; refers to rust on *P. secunda*]

≡*Caeoma* (*Aecidium*) *pyrolatum* Schwein., Trans. Am. Philos. Soc. N.S. 4: 294. 1834. [Based on uredinia]

=*Chrysomyxa ramischiae* Lagerh., Sven. Bot. Tidskr. 3: 26. 1909. [Refers only to rust on *Pyrola secunda*]

=*Melampsoropsis pyrolae* Arthur, Résult. Sci. Congr. Bot. Vienne, 1905, p. 338. 1906; N. Am. Flora, 7: 118. 1907. [Based on telia]

=*Aecidium ?pyrolae* DC., Flora Fr. 6: 99. 1815. [Refers to the rust on *P. secunda*]

=*Uredo pirolata* Körn., Hedwigia, 16: 28–29. 1877.

=*Caeoma pyrolae* Schltdl., Flora Berol., p. 122.

=*Trichobasis pyrolae* Berk., Outlines Br. Fungol. London, p. 332. 1860.

=*Peridermium conorum-piceae* (Reess) Arthur & Kern, Bull. Torrey Bot. Club, 33: 431. 1906.

≡*Aecidium conorum-piceae* Reess, Abhandl. Naturf. Ges. Halle XI: 102, 1869. Figs. 1–4. [*fide* Saccardo 1888]

=*Peridermium conorum* Thüm., Mitt. Forstl. Versuchswesen Oesterr. 2: 313. 1880.

=*Peridermium engelmanni* Thüm., Mitt. Forstl. Versuchswesen Oesterr. 2: 314. 1880.

=*Aecidium engelmanni* Dietel, in Engler & Prantl, Pflanzenfam. I(I): 79. 1897.

=*Aecidium conorum-abietis* Reess, Tageblatt Versammlung Deutscher Naturf., 42: 189. 1868.

Type: The correct type for *C. pirolata* is problematic. Hylander et al. (1953) list the following as the lectotype: on *Pirola* [*sic*] *rotundifolia*, Middle Europe. There is little information to trace the whereabouts of this collection. A tiny leaf fragment in PUR (4695) marked as part of type and "ex herb. Schweinitz. Phila." does bear telia, but a type from Europe would be more appropriate. Efforts are being made to trace exsiccati mentioned in Winter's description, including Rabenhorst's Fungi europaei et extraeuropaei.

Hosts and distribution: *Chrysomyxa pirolata* has a holarctic distribution, wherever the telial hosts occur: from Greenland and Iceland (Jørstad 1951), across Canada, south in the western mountains to New Mexico (Spaulding 1961) and in the east to Pennsylvania (Kern et al. 1929); Eurasia, including the United Kingdom (Wilson and Henderson 1966), Fennoscandia south to France (Jørstad 1934), Russia (Siberia, Kamchatka, Kuriles), India (Spaulding 1956), Pakistan (Kaneko 1993), China (Cummins and Ling 1950), and Japan (Hiratsuka et al. 1992). A report on *P. secunda* var. *elatior* (PUR 50507) is doubtful. Telial hosts are wintergreens in the genera *Moneses* (*M. uniflora* (L.) A. Gray and *M. reticulata* Nutt.) and *Pyrola* (*P. alpina*, *P. asarifolia* Michx., *P. bracteata* (Hook.) Haber, *P. dahurica* (Andres) Kom., *P. elliptica* Nutt., *P. grandiflora* Radius, *P. incarnata* Fisch., *P. media* Sw., *P. minor* L., *P. norvegica*, *P. picta* Smith (=*P. aphylla* Smith, *P. dentata* Smith), *P. renifolia* Maxim., *P. rotundifolia* L. (=*P. americana* Sweet), *P. secunda* L. (=*Orthilia secunda* (L.) House), *P. uliginosa* T. & G., *P. virens* Schweigg. (=*P. chlorantha* Swartz.).

The reported distribution on spruce is less extensive, probably because of the difficulty of observing and collecting the cones from tall trees and the ability of the rust to survive in Pyroloideae without host alternation. Spruce species reported as aecial hosts are *Picea abies*, *P. engelmannii*, *P. glauca*, *P. mariana*, *P. obovata*, *P. pungens*, *P. rubens*, *P. sitchensis*, and *P. smithiana*. It is not known on spruce in the United

Kingdom (Wilson and Henderson 1966), Iceland (Jørstad 1951), or Japan (Hiratsuka et al. 1992).

Description: On *Pyrola*, *Moneses*. Systemic, may cause discoloration or stunting. *Uredinia* small and densely crowded on lower surface of overwintered leaves or larger and scattered on both leaf surfaces (more commonly hypophyllous), petioles, bracts, and floral parts, 1/4–1 1/2 mm in diam, pulvinate, erumpent through epidermis, which splits and folds back around the sorus; surrounded by a delicate peridium of several layers of elongated cells with rounded apices, adheres to the underside of the epidermis after dehiscence. *Urediniospores* catenulate, ellipsoidal, ovoid, or polygonal to subglobose or globose, 15–35(–38) × 13–28 μm, melon-yellow (5A6) to deep orange (5A8); wall hyaline, thin, <1 μm thick, indistinct; warts crowded, annulate or with inconspicuous lateral ridges, rounded or polygonal in surface view, occasionally elongated and up to 10 μm long, with fine basal connections; *wall + warts* 1.2–3.3 μm thick. *Telia* hypophyllous and densely crowded on overwintered leaves of previous year, may cover entire leaf surface or when found with uredinia, they are mainly at the base and around the major leaf veins, pulvinate, circular or elongated, 1/4–1/2 mm in diam, confluent when mature, paler than uredinia, light orange (5A5 to 5A7), subepidermal, constricted at the base. *Teliospores* cuboid, irregularly oblong or ellipsoidal, 12–20 × 8–13 μm, wall thin, about 1 μm; chains of teliospores arise from a constricted base of sterile non-pigmented cells. *Basidia* four-celled, curved. *Basidiospores* globose to subglobose with a tiny apiculus, 5–10 × 4–10 μm.

On *Picea*. Systemic in cones, rarely young shoots. *Spermogonia* subepidermal, inconspicuous, shallow, indeterminate blisters on abaxial surface of cone scales, drying to pale brown vertical streaks or ridges distal to the aecia; in cross section, hymenium flat. *Spermatia* subglobose, ellipsoidal, or ovoid, 2.5–6 × 2.5–4 μm. *Aecia* at the base or higher on the cone scales, usually on the abaxial surface, occasionally adaxial, erumpent through the epidermis, bullate, variable in size, confluent, often occupying the entire width of the cone scale, smaller if on the adaxial surface. *Aeciospores* globose, subglobose, ellipsoidal, or ovoid, 21–42(–56) × 18–34 μm, deep orange (5A8 or 6A8);

wall indistinct; *warts* in surface view broad and pulvinate, round to polygonal, irregular, ovoid, or elongated, sometimes with small protuberances on upper surface, 1.6–4.1 μm high, annulate, with simple narrow ridges connecting warts basally. *Peridium* white, obvious in young sori, but evanescent and disintegrating during spore release; cells globose, subglobose, or ovoid, usually larger than aeciospores, 30–70 × 27–50 μm; wall thick, ornamentation variable, irregular fingerlike projections, which are longer at cell ends, sometimes a longitudinal groove.

Notes: *Nomenclature and history of name*

Various forms of the species name are found in the current literature: *C. pirolata*, *C. pyrolatum*, or *C. pyrolae*. None of the earliest descriptions of the rust fungus on *Pyrola* spp. was valid because telia were not included (De Candolle 1815; Schweinitz 1834; Körnicke 1877). Rostrup (1881) observed telia in Greenland, and gave a cursory description. Winter (1881) provided a more complete description of this stage, published in the same year. Rostrup refers to Winter's epithet, and therefore his description was likely published before Rostrup's. Therefore Winter's epithet, *C. pirolatum*, is the correct one, but requires the feminine form '*pirolata*' to agree with the gender of the genus name.

Life cycle

The connection of the rust on *Picea* and *Pyrola* was first suggested by Rostrup (1881), but confirmed by Fraser (1912, 1925), who successfully produced cone infections on *P. glauca* using germinating telia on *Pyrola* spp. Female strobili appear to be susceptible to infection when they are upright and open for pollination (Sutherland 1991), but detailed studies of the infection process have not been done. Preliminary studies show that basidiospores germinate on the cone scales to produce germ tubes that enter the host tissue directly between the epidermal cells (P.E. Crane, unpublished). Cones become systemically infected and spermogonia, and later aecia, form on all of the scales. In midsummer, infected cones are easily identified because they turn brown and open prematurely, releasing copious amounts of aeciospores. These are thought to produce new infections on the broadleaved hosts, but little is known about this process or of the

relative roles of aeciospores, urediniospores, and systemic hyphae in spreading the infection in these clonal plants. In early spring, uredinia, telia, or both types of sorus form on systemically infected *Pyrola* spp. and *Moneses* spp. The type of sorus that forms appears to be affected by environmental conditions, especially moisture (Crane and Hiratsuka 2000). Whereas telia are confined to leaves of the previous year, uredinia may also form on current-year leaves, floral parts, petioles, and bracts. The parts of the plant on which uredinia occur and whether they repeat during the growing season appears to depend greatly on the host species; it may also depend on environmental conditions (Crane and Hiratsuka 2000). *Pyrola asarifolia* plants observed in central Alberta seldom formed uredinia on current-year leaves, but specimens of this host from other areas sometimes had secondary uredinia. On this host species, flowers were seldom seen on infected plants, perhaps reflecting their reduced vigor; on several other *Pyrola* spp. and *M. uniflora*, however, uredinia were very common on flowers.

Morphology

Lagerheim (1909) described the rust on *P. secunda* as a separate species, *C. ramischiae*, based on the repeating uredinia on this host and the larger size of the uredinia. Jørstad (1936) favored a variety name, *C. pyrolae* var. *pyrolata* (Schw.) Jørstad, for the rust on *P. secunda*. The present study shows that some *Pyrola* hosts produce small crowded uredinia (<1/2 mm) in early spring, but larger scattered ones (>1/2 mm) later in the season on current-year leaves if uredinia repeat. On *Moneses uniflora*, they are large and scattered, regardless of whether early or late in the season. These characteristics are highly variable, and appear to depend on the host. Perhaps, as in several other rust genera, the size of uredinia increases with the susceptibility of the host (Roelfs and Martens 1988; Moltzan 1991; Stavely and Pastor-Corrales 1989). No consistent differences in other characters such as urediniospore morphology could be detected to support the separation of the rust on *P. secunda* or on other hosts. Comparison of urediniospore size from *P. secunda* with that from other hosts did not support earlier claims that they are smaller on *P. secunda* (Arthur 1934) (Table 2.4).

Table 2.4. Comparison of mean urediniospore size of *C. pirolata* on several hosts

Host (No. specimens, No. spores)[a]	Range, μm	Mean, μm
Moneses uniflora (10, 50)	18–32 × 14–26	24.8 × 18.3
Pyrola asarifolia (7, 50)	18–35 × 14–22	24.2 × 18.3
P. minor (2, 20)	20–30 × 17–23	23.9 × 19.1
P. rotundifolia (4, 44)	19–34 × 16–26	26.2 × 19.0
P. secunda (4, 50)	17–31 × 16–23	25.1 × 19.1
P. virens (8, 50)	(18)21–34 × 15–22	26.8 × 18.8

[a]Spores were selected randomly from several collections. Numbers in parentheses after host name are number of collections and total number of spores, respectively, used to calculate the means.

Field evidence, however, suggests that there may be different races of *C. pirolata* specific to certain telial hosts (Savile 1950). At Smoky Lake, in central Alberta, infection of *P. asarifolia* was common, but adjacent *P. secunda* were unaffected. Similarly, W.G. Ziller noted, on a collection of infected *P. minor* from Lake Louise, Alberta (DAVFP 10908), that adjacent *M. uniflora* was entirely free of rust. DNA comparisons, such as PCR fragment analysis or sequencing studies, or inoculation experiments are needed to answer this question.

Variability in spore surface morphology (shape and annulation of warts) was observed among collections of *C. pirolata*. Elongated warts often occurred among more rounded ones on the same urediniospores or aeciospores; in several samples, elongated warts predominated (Fig. 2.17, F–H) . These were from widely separated locations (Ontario, DAVFP 6067; Quebec, PUR 63334; Northwest Territories, CFB 6755; Alberta, CFB 4281, 8023), and this character could not be correlated with a particular host. Therefore the significance of this variant is unknown. Although aeciospore warts were consistently annulate, this was not the case for urediniospores. Well-defined warts with annuli that could be seen by light microscopy were only observed in specimens collected from late July to October, i.e. from secondary uredinia on current year leaves. More shallow, less defined warts with inconspicuous lateral ridges were characteristic of spores from primary sori on leaves of the previous year.

Two specimens of *C. pirolata* on young spruce shoots rather than cones are deposited in DAVFP. Both were from northwestern British Columbia and one originated in a nursery. The aeciospores were characteristic of *C. pirolata*, and there appeared to be spermogonia at the base of adjacent needles, but this could not be confirmed microscopically. The possibility that there are races of this rust capable of damaging spruce seedlings needs to be investigated.

Chrysomyxa pirolata exhibits a number of morphological characteristics that are different from most other members of the genus: the broad, pulvinate warts; peridial cells with a sporelike shape and ornamentation; possibly indeterminate spermogonia; and teliospores borne on elongated sterile basal cells. Several other species presently

included in the genus have sterile basal cells in the telia (see Introduction). It is not known whether they share a common ancestry or whether this character has arisen independently. The spermogonia of *C. pirolata*, while subepidermal like other species of *Chrysomyxa*, appear to be indeterminate (group IV, type 8, Hiratsuka and Cummins 1963), rather than determinate and flask-shaped (group I, type 1 or 2). Studies of the ontogeny of these structures is necessary to determine whether they are truly indeterminate, or whether smaller discrete sori coalesce as they mature. In Uredinales, characters such as the spermogonium type and the telial morphology are considered significant at the genus level (Hiratsuka and Cummins1963; Hiratsuka and Hiratsuka 1980). The above characters may indicate that *C. pirolata* does not belong in this genus.

The only other systemic cone rust occurring on spruce in North America is *C. monesis*. It can be distinguished by light microscopy from *C. pirolata* by its aeciospore and urediniospore ornamentation (see Notes under *C. monesis*). Other rusts that are occasionally found on spruce cones in North America are *C. ledicola* and *Pucciniastrum americanum* (Farl.) Arth. Both produce localized, not systemic, infections. The subcuticular spermogonia of the latter distinguish it from a chrysomyxa. In Eurasia, the rust *Thekopsora areolata* (Fr.) Magnus also occurs systemically in spruce cones. It differs from *C. pirolata* in its small brown dome-shaped aecia, subcuticular spermogonia, aecia mainly on the inner rather than outer surface of the cone scales, and brown to gray aeciospores (Roll-Hansen 1965; Kuprevich and Tranzschel 1957).

Impact

Interest in *Chrysomyxa pirolata* has increased in recent years because of the establishment of spruce orchards to produce high-quality seed for reforestation (Sutherland 1990). Spruce trees produce large cone crops infrequently and therefore high disease levels can result in economic loss. The incidence of cone rust varies greatly from year to year, but in certain areas affects nearly the entire crop in both natural forests and seed orchards in North America and Europe (Jørstad 1936, 1951; Roll-Hansen 1967; Singh and Carew 1990; Sutherland 1990). The disease significantly reduces seed yield, seed germination, and seed weight; it may also interfere with seed dispersal and result in

abnormal germination (Jørstad 1936; Nelson and Krebill 1970; Singh and Carew 1990; Sutherland 1981, 1990).

Specimens examined: On *Moneses uniflora*. **CANADA: Alberta:** Pigeon Mt. Lookout, 17 Aug 1965, J.M. Powell (CFB 6860); Red Earth Cr. Trail, Banff Natl. Park, 5 Jul 1997, P.E.C. (CFB 22076); Devil's L., Banff Natl. Park, 5 Jul 1907, Holway (PUR 4618). **British Columbia:** Lillooet, 11 Jun 1961, S.S. Holland (DAVFP 13381); Muncho L., 28 Jul 1962, A.F. Szczawinski (DAVFP 14481). **Northwest Territories:** Ft. Smith, 2 Jul 1950, W.J. Cody & C.C. Loan (PUR 53206, ex DAOM 25762). **Quebec:** Great Whale R., 16 Aug 1939, E.C. Abbe et al. (PUR 63333). **USA: Michigan:** Rock Harbor, Isle Royale, 30 Jun 1930, A.H. Povah (DAVFP 19585). **Montana:** L. Josephine, 8 Jul 1919, P.C. Standley (PUR 4615). **New Mexico:** Santa Fe Canyon, 16 Jul 1916, J.N. O'Byrne (PUR 4616).

On *Pyrola aphylla*. **CANADA: British Columbia:** McBride, 23 Aug 1954, J. Grant (DAVFP 9395).

On *P. asarifolia*. **CANADA: Alberta:** Kananaskis For. Exp. Stn., 23 Jun 1961, J.A. Baranyay (CFB 4867); 17 mi SE of Rocky Mountain House, 10 Sep 1965, G.J. Smith (CFB 6758); 2 mi NW of Miette Hot Springs, 26 May 1969, J.A. Parmelee & Y.H. (CFB 20308, ex DAOM 130360). **British Columbia:** Hope, 29 May 1950, W.G.Z. (WINF(M) 3485, ex DAVFP 6063). **Yukon Territory:** Dawson City, 15 Jun 1969, J. Susut (CFB 8884). **USA: Colorado:** 1902, F. Clements (PUR 4623). **New Mexico:** Winsor Cr., Pecos Natl. For., 29 Jun 1908, P.C. Standley (PUR 4624). **Wyoming:** N. French Cr., Medicine Bow Mts., Carbon Co., 7 Jul 1950, W.G. Solheim (PUR 52377).

On *P. dentata*. **CANADA: British Columbia:** Echo L., 12 mi E of Lumby, 4 Oct 1965, J. Grant (DAVFP 15949).

On *P. ?elliptica*. **CANADA: British Columbia:** Cinema, 16 Sep 1955, W.G.Z. (DAVFP 9750).

On *P. grandiflora*. **CANADA: Northwest Territories:** Tuktoyaktuk, 69°27' N, 133°02' W, 5 Jul 1963, J.A. Parmelee (CFB 8706, ex DAOM 115743). **Quebec:** Great Whale R., 21 Aug 1939, Abbe et al. (PUR 63334).

On *P. minor*. **CANADA: Alberta:** Kananaskis For. Exp. Stn., 24 Jul 1969, J.A. Parmelee (CFB 20410, ex DAOM 130361); Lake Louise, 5 Jul 1958, W.G.Z. (DAVFP 10908). **British Columbia:** Vernon, 25 Jun 1965, J. Grant (DAVFP 16733). **Yukon Territory:**

Dezadeash, 1 Aug 1962, W.G.Z. (DAVFP 14629). **SWITZERLAND:** Canton of Neuchatel, 1 Jul 1939, E. Mayor (DAVFP 11961).

On *P. rotundifolia*. **SWITZERLAND:** Canton of Neuchatel, 29 May 1944, E. Mayor (DAVFP 11963). **USA: Connecticut:** New Haven, May 1894, W.C. Sturgis (PUR 4689). **New Hampshire:** Chocorua, May 1908, W.G. Farlow (PUR 4655). **New York:** no date, Torrey (PUR 4695, Type of *Aecidium pyrolatum*); Albany, May 1876, C.H. Peck (PUR 4694).

On *P. secunda*. **CANADA: British Columbia:** Chancellor Peak Campground, Yoho Natl. Park, 20 Jul 1966, V.B. Patterson (CFB 7205); Prince George, 19 Sep 1951, N.T. Engelhardt (DAVFP 7095). **Northwest Territories:** 32 mi SE of Arctic Red R., E. Gautreau, (CFB 8202). **SWITZERLAND:** Canton of Neuchatel, 27 May 1919, E. Mayor (DAVFP 11957). **SWEDEN:** Upland, Rådmanto, D. Palm, det. Lagerheim, July 1908 (K, type of *C. ramischiae*). **USA: New Mexico:** Brazos Canyon, Rio Arriba Co., 31 Aug 1914, P.C. Standley & H.C. Bollman (PUR 4680).

On *P. secunda* var. *elatior*. **GUATEMALA:** Dept. Quyaltenango, Volcán Zunil, 22 Jan 1940, J.A. Steyermark (PUR 50507, unconfirmed as *C. pirolata*).

On *P. virens* (*P. chlorantha*). **CANADA: Alberta:** Strachan, 27 Jun 1953, J. Kuijt (CFB 707); Vermilion Pass Rd., 3.2 mi W of Eisenhower Jct., Banff Natl. Park, 14 Jun 1961, R.T. Ogilvie (CFB 4852); 53 mi NW of Worsley, 12 Aug 1965, C.R. Layton (CFB 6673); Honeymoon Cr., Jasper Natl. Park, 12 Aug 1998, Y.H. (CFB 22191). **British Columbia:** Kamloops, 21 Jun 1949, W.G.Z. (DAVFP 4740); Clinton, 30 Jun 1950, W.G.Z. (DAVFP 6065); Martha Cr., 28 Jun 1952, G.P. Thomas (DAVFP 7796); Manning Park, 26 Aug 1952, J. Kuijt, (DAVFP 7875; 27 Aug 1952, DAVFP 8063); Atlin, 8 Aug 1962, A.F. Szczawinski (8 Aug 1962). **Northwest Territories:** 8 mi E of Yellowknife, 20 Jul 1965, E. Gautreau (CFB 6755); 11 mi S of Frank Channel, 10 Jun 1966, E. Gautreau (CFB 7245); 33 mi N & 1 mi W of Fort Providence, 7 Jun 1966, E. Gautreau (CFB 7257). **USA: Wyoming:** Donoho Pt. in Jackson L., Grand Teton Natl. Park, 3 Aug 1955, W.G. Solheim (PUR 54988).

On *Pyrola* sp. **CANADA: Alberta:** 30 mi SW of Sundre, 26 May 1966, G.J. Smith et al. (CFB 6945); 15 km N of Carson-Pegasus Prov. Park, 19 May 1996, P.E.C. (CFB 22020); Fish L. campground, Nordegg, T. Lumley (CFB 22021). **Ontario:** Chalk River, 31 May 1950, E. Eggertson (DAVFP 6067).

On *Picea abies*. **SWITZERLAND:** Canton of Neuchatel, 10 Jun 1937 (DAVFP 11954).

On *P. engelmannii*. **CANADA: Alberta:** Lynx Cr., Blairmore, 4 Sep 1952, D.E. Etheridge (CFB 146); 11 mi N of Coleman, 24 Aug 1960, J. Watson (CFB 4281); Crowsnest campground, 20 Jul 1961, E. Gautreau (CFB 4650); 24 mi S of Banff, 8 Aug 1964, J. Petty (CFB 6213); 15 mi NW of Waterton, 24 Aug 1967, G.J. Smith (CFB 8023). **British Columbia:** Silverton, 1 Aug 1985, R. Turnquist (DAVFP 23227); Invermere, 1 Aug 1985, H.P. Koot (DAVFP 23229).

On *P. glauca*. **CANADA: Alberta:** 4 mi W & 9 mi N of Hines Cr., nr. Eureka R., 11 Jul 1968, R.M. Caltrell (CFB 8381); 10 mi E of Elkwater, 19 Aug 1967, G. Bigalow (CFB 7918); Pine Ridge Tree Nursery, Smoky Lake, 14 Aug 1996, P.E.C. (CFB 22049). **Manitoba:** Wasagaming, 18 Aug 1967, D. Shepherd (WINF(M) 7907); Paint L., Duck Mt. Forest Reserve, 26 Sep 1967, R.J. Kovach & F.B. Armitage (WINF(M) 9064). **Northwest Territories:** 10.5 mi N of Fort Providence, 21 Aug 1962, G. Kleinhout (CFB 5295). **Yukon Territory:** 9 mi SE & 17.5 mi S of Whitehorse, 14 Aug 1968, J. Susut (CFB 8671); 10.9 mi E of Teslin, 21 Aug 1969, J. Susut (CFB 9029). **USA: Minnesota:** Grand Rapids, 10 Jul 1984, J. Campbell (DAVFP 23135).

On *P. mariana*. **CANADA: British Columbia:** Prince George, 14 Aug 1951, C.B. Cottrell (DAVFP 7066).

On *P. pungens*. **CANADA: Alberta:** 4.5 mi E of Faust, 27 Jul 1967, C. Layton (CFB 8104). **British Columbia:** Smithers, 26 Jul 1961, E.G. Harvey (DAVFP 12750).

On *P. sitchensis*. **CANADA: British Columbia:** Atlin, 30 Aug 1963, J. Holms (DAVFP 15466); Terrace, 11 Sep 1956, W.G.Z. (DAVFP 10161); Terrace, 2 Aug 1968, E.V. Morris (DAVFP 18256, on shoots). **USA: Alaska:** Cordova, 8 Sep 1950, J.H. Clough (DAVFP 19582).

On *P. smithiana* (Wall.) Boiss. **PAKISTAN:** Bial Camp, Fairy Meadow, Diamar, Gilgit, 7 Oct 1991, S. Kaneko (CFB 22176, ex TFM-FPH 2139).

On *Picea* sp. **CANADA: British Columbia:** Prince George, 18 Aug 1988, R. Massey, (DAVFP 23771, on shoots).

Chrysomyxa reticulata P.E. Crane, sp. nov. **Fig. 2.19, A–I**

=*Chrysomyxa ledi* var. *rhododendri* (de Bary) Savile, Can. J. Bot. 33: 491. 1955. *p.p.*

Type: Tunnel Trail, S of Ribbon Cr. Trail, Kananaskis, Alberta, 1 Jun 1998, P.E. Crane (Holotype DAOM, Isotypes PUR, DAVFP, CFB 22183).

Hosts and distribution: This rust has been found on *L. groenlandicum* and *L. decumbens* in both eastern (Nova Scotia) and western Canada (Alberta, British Columbia), the northeastern (Wisconsin) and northwestern United States (Washington, California). In Washington and California it has infected cultivated rhododendrons in nurseries. The aecia are so far known only from artificial inoculation of *Picea glauca*.

Description: In *Ledo* et *Rhododendro*. *Uredinia et telia* hypophyllosa in foliis anni superioris. *Uredinia* in circulis, per epidermim erumpentia, circularia, <1/8–1/2 mm lata, per mediam partem fere globosa, cum peridio delicato et inconspicuo de una propagine vel duabus cellularum irregularium vel leviter tunicatarum. *Urediniosporae* globosae ad subglobosae, aliquando ellipsoideae vel ovoideae, 15–24 (–26) × 12–21 μm, pallide aurantiaco-flavae, mox ad album pallescentes; tunica hyalina, levissima, 0.4–0.8 μm; verrucae separatae, congregatae, cylindricae, altitudine aequa, annulatae, cum summis latis et inaequalibus; latus alterum sporae velatum ab area longitudinali, lata, reticulata; *tunica et verrucae* 0.8–2.5 μm crassae. *Telia* cerea, pulvinata, subepidermalia, fusciora quam uredinia, aurantiaca, rare confluentia, circularia, 1/4–1/3 mm diam, per mediam partem lenticularia. *Teliosporae* catenulatae, cuboideae ad oblongae, 12–19 × 8–12 μm, cum tunica levi et hyalina, <0.8 μm. *Basidiosporae* ellipsoideae, subglobosae vel globosae, cum apiculo exiguo et angusto, 5–8 × 5–8 μm.

In *Picea*. *Spermogonia et aecia* in acuibus arboreis huius anni. *Spermogonia* amphigenosa, subepidermalis, pallide aurantiaca, fuscans ad auro-brunnea quando maturat; per mediam partem plus/minus globosa, 100–110 μm lata × 80–130 (–150) μm alta. *Aecia* amphigenosa, parvissima. *Aeciosporae* globosae ad subglobosae, saepe cum extremo plano, pars tegamenti levis et longitudinalis cum centro concavo, 15–18 (–21) × 14–18 μm, clare aurantiacae; tunica hyalina, tenuis; *tunica et verrucae* 1.6–2.5 μm crassae. *Peridium* tenuissimum et delicatum, non persistens; cellulae imminentes, multo

largiores quam sporae, 34 × 30 μm, facile separant, in externa parte concavae et leves, in parte interna paulo concavae et rugosae.

Etymology: Named for the reticulate ornamentation on part of the urediniospores.

On *Ledum* and *Rhododendron*. *Uredinia and telia* hypophyllous on leaves of previous year. *Uredinia* in groups, erumpent through the epidermis, circular, <1/8–1/2 mm wide, in cross section almost globose, with a delicate, inconspicuous peridium of one or two layers of irregularly shaped, thin-walled cells. *Urediniospores* globose to subglobose, occasionally ellipsoidal or ovoid, 15–24(–26) × 12–21 μm, pale yellow orange, soon fading to white; wall hyaline, very thin, 0.4–0.8 μm; warts separate, crowded, cylindrical, even in height, annulate, with broad, uneven tops; one side of spore covered by a broad longitudinal reticulate area; *wall + warts* 0.8–2.5 μm thick. *Telia* waxy, pulvinate, subepidermal, darker than uredinia, orange (5B8), seldom confluent, circular, 1/4–1/3 mm in diam, in cross section lenticular. *Teliospores* catenulate, cuboid to oblong, 12–19 × 8–12 μm, with a thin hyaline wall, <0.8 μm. *Basidiospores* ellipsoidal, subglobose or globose, with a tiny narrow apiculus, 5–8 × 5–8 μm.

On *Picea*. *Spermogonia and aecia* on current-year needles. *Spermogonia* amphigenous, subepidermal, pale orange, darkening to golden brown with age; in cross section ± globose, 100–110 μm wide × 80–130(–150) μm high. *Aecia* amphigenous, very small. *Aeciospores* globose to subglobose, often with one flat end, part of a smooth longitudinal cap with a central groove, 15–18(–21) × 14–18 μm, light orange (5A5); wall hyaline, thin; *wall + warts* 1.6–2.5 μm thick. *Peridium* very thin and delicate, not persistent; cells overlapping, much larger than spores, 34 × 30 μm, separate easily, on outside concave and smooth, on inside shallowly concave and rugose.

Notes: *Chrysomyxa reticulata* has the smallest spores of any known species in the genus. In addition to North American and European species, spore size was compared with records of Asian species to confirm that this newly found rust did not originate there. *Chrysomyxa reticulata* was first found on a specimen of *Ledum groenlandicum* from Nova Scotia (PUR 4866). Although most uredinia on the leaves of this sample were of *C. nagodhii*, a few pustules on one leaf petiole bore smaller spores with different

surface ornamentation. Both uredinia and telia were seen on a sample of the same host from Wisconsin collected in 1893. The early date of this collection as well as the widespread distribution (see Specimens examined), including locations in northern and western Alberta and northern British Columbia, far from introduced rhododendrons, suggest that *C. reticulata* is native to North America, and not imported. It was likely overlooked earlier because of its occurrence on the same host as *C. nagodhii*. Although *C. reticulata* appears to be a minor component of the rust flora on *Ledum* spp., its recognition is horticulturally important because it occurs on cultivated rhododendrons. It has been assumed that the first outbreaks of rust on cultivated rhododendrons on the west coast of North America during the 1950's originated from infected nursery stock imported from Europe (Gould et al. 1955; Gould 1966; Savile 1955, 1973). On the contrary, the present study shows that these outbreaks were likely caused by *C. reticulata* that spread to nurseries from native *Ledum* spp. This is supported by the following evidence. (1) Samples of both *Ledum* and cultivated rhododendrons from Long Beach, Washington, one of the first sites of these outbreaks, are preserved in PUR. (2) The unusually small size (for *C. rhododendri*) of the urediniospores. (3) The warm coastal habitat of Washington would be unusual for *C. rhododendri*, which is native to far northern and alpine habitats in North America and Europe. *Chrysomyxa reticulata* appears to have a much more widespread habitat distribution. (4) The morphology and spore size is identical for the rusts on both host genera (Figs. 2.9, 2.19).

It should be noted that not all rusts found on cultivated rhododendrons in North America are *C. reticulata*. *Chrysomyxa nagodhii* was also identified on one specimen from Vancouver Island; another plant, with a notation that it was imported from Tibet, appeared to be *C. rhododendri*. Scanning electron microscopy has elucidated the unique morphology of the spores of *C. reticulata*, and rusts appearing on cultivated rhododendrons should be identified by microscopic examination. Planting rhododendrons near native *Ledum* spp. should be discouraged.

On cultivated rhododendrons, *C. reticulata* does not appear to produce telia; on *Ledum*, telia were found only on the type material, the above-mentioned Wisconsin

sample, and possibly one from northern British Columbia (DAVFP 20261). At the type locality in Kananaskis, Alberta, this rust was producing uredinia earlier than either *C. ledicola* or *C. nagodhii*, which were also present in the same area. Gelatinous sori among the uredinia developed into telia when the infected leaves were placed in a moist chamber for several days. When mature, these telia were used to infect spruce, thus elucidating the entire life cycle. Naturally infected spruce were not found, however, in the Kananaskis location later in the growing season. Perhaps this rust rarely produces telia in nature. I considered the possibility that the telia used for inoculation were actually of *C. nagodhii*. The aecial state produced on spruce needles, however, differed in several ways from *C. nagodhii*: a delicate, ephemeral peridium without coarse striate side walls, pale rather than deep orange spores, smaller aeciospores (Fig. 2.9) with a smooth longitudinal cap having a central groove (Fig. 2.19, F), and narrower intercellular hyphae (3–4 μm compared with 5–6 μm in *C. nagodhii*).

It is possible that *C. reticulata* has been introduced to other areas of the world by the importation of rhododendrons from the United States. Bennell (1985) noted that rust introduced to Scotland on planting stock from the western United States in 1980 infected rhododendron species that were resistant to the *C. rhododendri* rust already present; other species that were normally highly susceptible did not become infected. The rust was assumed to be a new pathotype of *C. rhododendri*. It is also possible that the introduced rust was *C. reticulata*.

Specimens examined: On *Ledum decumbens*. **CANADA: Alberta:** Unnamed L., 5 Jul 1966, J. Susut & J. Petty (CFB 7334). **British Columbia:** Muncho L., 9 Jun 1972, C.S. Wood (DAVFP 20261).

On *L. groenlandicum*. **CANADA: Alberta:** Tunnel Trail, S of Ribbon Cr. Trail, Kananaskis, 1 Jun 1998, P.E.C. (CFB 22183, Type); Kananaskis For. Exp. Stn., Ribbon Cr., 6000 ft, 9 Oct 1958, R.T. Ogilvie (CAFB). **British Columbia:** Daisy Lake, 13 Oct 1924, J.S. Boyce (PUR 4881); Ft. St. John, 29 Jun 1965, R.O. Wood (DAVFP 16754). **Nova Scotia:** Pictou, 26 Jun 1909, W.P. Fraser (PUR 4866); Numa Cr. Trail, Kootenay Natl. Park, 28 Jul 1965, V.B. Patterson (CFB 6809). **USA: Wisconsin:** Three Lakes, Jul 1893, J.J. Davis (PUR 4877).

On *Rhododendron odoratum*. **USA: Washington:** Long Beach, 4 Apr 1954, C.J. Gould (PUR 53445).

R. racemosum × *ciliatum*. **CANADA: British Columbia:** Saanich, Vancouver Is., 18 Jul 1958, W.G.Z. et al. (DAVFP 10764); 17 Jul 1958, W.G.Z. & G.S. Brown (DAVFP 10762).

On *Rhododendron* sp. **CANADA: British Columbia:** Vancouver, 27 Nov 1998, Y.H. (CFB). **USA: California:** Oct. 1962 (PUR 57912, 57913). **Washington:** Long Beach, 3 Aug 1955, Wheeler et al. (PUR 54533).

On *Picea glauca*. **CANADA: Alberta:** Edmonton, 9 Jul 1998, P.E.C. (CFB 22182, from inoculation).

Chrysomyxa rhododendri de Bary, Bot. Z. 37: 809. 1879. **Fig. 2.20, A–H**

≡*Chrysomyxa ledi* var. *rhododendri* (de Bary) Savile, Can. J. Bot. 33: 491. 1955.

=*Uredo rhododendri* DC., Flore Fr. 6: 86. 1815.

≡*Chrysomyxa ledi* var. *rhododendri* (DC.) Savile,Can. J. Res., C, 28: 325. 1950. [*nom. nud.*]

≡*Coleosporium rhododendri* (DC.) J. Schröt. (subgen. *Melampsoropsis*), in Cohn, Beitr. Biol. Pflanzen, Band I, Heft 3, Pt. II, p. 56. 1879. [Based on uredinia]

=*Caeoma rhododendri* Link, Spec. Plant. II, p. 16. 1825.

=*Caeoma piceatum* Link, in Linné, Spec. Plant. VI, 2: 62, 1825. *p.p.*

=*Melampsora rhododendri* Thüm., 1880 [*fide* Hiratsuka et al. 1992]

=*Melampsoropsis rhododendri* Arthur, Résult. Sci. Congr. Bot. Vienne, 1905, p. 338. 1906.

=*Aecidium abietinum* Alb. & Schwein., Consp. Fung., p. 120. 1805. *p.p.* [combined aecia of *C. ledi* & *C. rhododendri* (Sydow 1915)]

=*Erysibe rhododendri* Wallr., Flora Cryptogam. Ger. II, No. 1622, p. 199. 1833. [Based on uredinia]

Lectotype: On *Rhododendron hirsutum* L., Schächenthal in Uri, Switzerland, de Bary (syntype K!).

Hosts and distribution: *Chrysomyxa rhododendri* has a wide distribution, and is endemic to Europe, Greenland, far northern North America, Siberia, China, Korea, Taiwan, and Tibetan East Himalayas. The aecial stage is found on *Picea abies*, *P. sitchensis* (Japan, Britain, Europe, not in North America); *P. jezoensis* Carr., *P. glehnii* Mast. (Japan); *P. brachytyla* (China), *P. obovata* Ldb. Numerous species of rhododendron, both wild and cultivated, have been documented as hosts to *C. rhododendri*. Some of these reports may not be accurate because of confusion with other species. For example, many reports of *C. rhododendri* on cultivated rhododendrons in North America are probably *C. reticulata* or *C. nagodhii* (see Notes under *C. reticulata*). Therefore mention will be made of only native hosts in Europe (*R. ferrugineum* L. and *R. hirsutum* and their hybrids) and North America and Greenland (*R. lapponicum* (L.) Wahlenb.). For hosts affected in Asia and in cultivation in Europe and North America, the reader is referred to lists elsewhere (Gäumann 1959; Leppik 1974; Bennell 1985; Farr et al. 1996). Importation of horticultural varieties of rhododendrons has resulted in spread of this rust to New Zealand and Australia (Bennell 1985).

Description: On *Rhododendron*. *Uredinia and telia* hypophyllous on leaves of previous year; *uredinia* also on leaf petioles, fruit pedicels, and twigs. *Uredinia* scattered, partially or completely covering underside of some leaves but absent from others, erumpent through epidermis, round, pulvinate, 0.2–0.7 mm wide, larger on twigs, flat-bottomed in cross section, with an inconspicuous peridium of collapsed, thin-walled cells. *Urediniospores* mostly ellipsoidal or ovoid, occasionally subglobose or globose, one or both ends slightly flattened or with a small cap, part of a shallow longitudinal stripe containing shallow, irregular bumps, 18–32(–36) × 14–22 μm, apricot color (5B6); wall hyaline, <1 μm thick; *wall + warts* 1.2–2.9 μm thick. *Telia* in groups, confluent, erumpent through epidermis, larger and more irregular in shape than uredinia, up to 1 mm long. *Teliospores* cuboid to ellipsoidal or irregular, 20–28 (–34) × 12–21 μm, wall colorless, thin, smooth. *Basidiospores* globose to subglobose, 8–10 × 8 μm, with a small apiculus.

On *Picea*. *Spermogonia and aecia* on yellowed zones of current-year needles, causing premature defoliation. *Spermogonia* prominent, round or elongated on needle surface, amphigenous; in cross section, hymenium broad and flat to shallowly concave, 140–220 μm wide × 110–150 μm high. *Spermatia* globose to subglobose, 2.5–3.7 × 2.0–3.3 μm. *Aecia* amphigenous, variable in size, 0.3–1.3 mm wide, single or confluent. *Aeciospores* variable in shape from globose to ellipsoidal or ovoid, with one or both ends flat or with a small delicate cap, part of an indistinct longitudinal stripe containing irregular shallow bumps, 18–30 × 16–22 μm, light orange (5B5 or 5A5); wall hyaline, *wall + warts* 2.0–3.3 μm thick. *Peridium* delicate, shredding at maturity, but persistent; on outside, cells shallowly concave, smooth; on inside, convex with shallow warts, sometimes appearing labyrinthine; lateral walls narrow (~2 μm), striate.

Notes: *Morphology and life cycle*

Unlike the *Ledum* rusts, which are morphologically different between Europe and North America, *C. rhododendri* on native rhododendrons in Europe and northern Canada is remarkably similar. The urediniospores are fairly uniform in both spore size and surface morphology. Whereas telia are abundant on European native rhododendrons, however, they have not been reported in North America on *R. lapponicum*, and therefore the rust is unknown on spruce on this continent. Whether this suggests a different race of the rust (Savile 1950) or merely a response to a different host or climate is unknown. In Britain, where *C. rhododendri* is an introduced species, the stage that develops also varies with the host, with uredinia most common. On *R. ponticum*, though, telia predominate (Wilson and Henderson 1966). Inoculation experiments suggest that the host strongly influences the stage that develops. When cultivated rhododendrons were inoculated with the same inoculum under uniform conditions, uredinia only, telia only, or both uredinia and telia were produced, depending on the host (Bennell 1985).

The connection of the aecial and telial stages of *C. rhododendri* was proven by de Bary (1879) and confirmed by Klebahn (1905). In subalpine regions of Europe, teliospores appear on native rhododendrons in early summer during flowering of the host. Basidiospores infect young spruce needles, producing spermogonia within 10 days, and

later aecia, from late July until early September. Aeciospores infect mature rhododendron leaves and form uredinia. The next spring, telia develop from overwintering mycelium (Gäumann 1959).

On cultivated rhododendrons, however, the behavior of *C. rhododendri* may vary considerably, depending on host phenology, prevailing climate, and probably the pathotype of the rust involved. Thus in Britain, five major life cycle patterns have been recognized: a single annual cycle of urediniospores, recycling of uredinia in the growing season, year-round sporulation in sheltered conditions, alternation to spruce along with any of the previous patterns, and obligatory alternation (Bennell 1985).

De Bary (1879) clearly illustrated the major differences between the peridial cells of *C. ledi* and *C. rhododendri* and this study confirms his observations. The wide (3–6 μm or more) coarse striate margins of the peridial cells of *C. ledi* are much more obvious than the more delicate lateral margins (2 μm) of *C. rhododendri*. The mean urediniospore size of the two species is very similar, but the aeciospores of *C. ledi* tend to be longer than those of *C. rhododendri* (Fig. 2.9, A, B). Whereas *C. ledi* spores have a narrow groove and long tapered warts, *C. rhododendri* has an indistinct smooth or irregular longitudinal stripe and cylindrical warts. The habitat differences (*C. rhododendri* occurs mainly in subalpine regions in Europe, *C. ledi* at lower elevations farther north) also support the separation of these two taxa as distinct species. *Chrysomyxa roanensis* and *C. piperiana*, two other rhododendron rusts endemic to North America, both have longer spores than *C. rhododendri*.

A specimen examined from China was consistent with the species description presented here. The samples from Japan, however, had a different surface ornamentation, with warts very crowded and often joined laterally at the top. They may be a distinct species; therefore these samples were not used in compiling the description.

Impact

During rainy summers, *C. rhododendri* may cause serious damage to *P. abies* in Europe (Biraghi 1954; Bohmer et al. 1998). Severe infection may also occur in Britain

on *P. abies* (Gardener's Chronicle 1943), whereas *P. sitchensis* appears to be more resistant (Wilson and Henderson 1966). Recent studies (Bauer et al. 2000) in Austria show that afforestation and regeneration of Norway spruce is seriously impaired in regions of frequent attack by *C. rhododendri*. The fungus reduces photosynthetic capacity of infected trees, reducing carbohydrate production, and significantly decreasing radial growth in heavily infected trees (Oberhuber et al. 1999).

Chrysomyxa rhododendri is the most widely distributed rust of rhododendrons in cultivation (Bennell 1985). It disfigures and discolors ornamental rhododendrons, reducing their value. In cultivation the rust has been able to attack over 50 new host species not known to be affected in their wild habitats. Hybrids between susceptible and non-host species are often susceptible (Bennell 1985). Although *C. rhododendri* probably exists in North America on cultivated rhododendrons, transfer of native rusts (*C. nagodhii*, *C. reticulata*, *C. ledicola*) to these hosts also occurs. The identity of rhododendron rusts on this continent should be verified by careful microscopic examination.

Specimens examined: On *Rhododendron ferrugineum*. **SWITZERLAND:** Tr. to Nair L., St. Moritz, Engadine, 18 Aug 1998, R. Berndt (CFB 22180); Canton of Valais, 20 Aug 1954, E. Mayor (DAVFP 11964). **AUSTRIA:** Tirol, Brixen, 29 Jul 1891, P. Dietel (PUR 530).

On *R. hirsutum*. **AUSTRIA:** Hohentauern, Pfandolseharte, 29 Jul 1891, P. Dietel (PUR F532).

On ×*R. intermedium* Tausch. (=*R. hirsutum* × *R. ferrugineum*). **AUSTRIA:** Tirol, Gschnitzthal (?), 2 Aug 1891, P. Dietel (PUR F533, Sydow Uredineen).

On *R. kaempferi* Planch. **JAPAN:** Agematsu (Kiso), Shinano, 8 Aug 1931, N. Hiratsuka (PUR F12695); Yamaguchi-mura, Chikuzen, 29 Aug 1931, O. Ishiuchi (PUR F12696).

On *R. keleticum* Balf.f & Forrest. **UNITED KINGDOM:** Sevenoaks, Kent, 30 Sep 1948, W.N. Wheeler (PUR 11729).

On *R. kiusianum* Makino. **JAPAN:** Kirishima Hotsprings, Kirishima Mts., Osumi, 25 Oct 1939, N. Hiratsuka (PUR F12694).

On *R. lapponicum*. **CANADA: British Columbia:** Summit Pass, mi 392 Alaska Hwy., 10 Aug 1962, W.G.Z. (DAVFP 14606). **Manitoba:** Fort Churchill, 4 Sep 1950, D.B.O.S. &

W.B. Schofield (PUR 53201, ex DAOM 24977). **Northwest Territories:** S. Nahanni R. area, Mackenzie Mtns., 61°18' N 124°06' W, 26 Jun 1970, G.W. Scotter (CAFB 3474); Indin L., Dist. Mackenzie, 64°17' N 115°12' W, 12 Aug 1949, W.J. Cody & B.J. McCanse (PUR 52021, ex DAOM 23509).

On *R. mucronulatum* Turcz. var. *ciliatum* Nakai. **KOREA:** Shayu-rei, Mosan-gun, Kanhoku, 6 Jul 1939, N. Hiratsuka (PUR F12697).

On *R. myrtifolium* Schott & Kotschy. (=*R. minus* × *R. hirsutum*). **HUNGARY:** Siebenbürgen, Jul 1883, Linhart (PUR F535).

On *R. roylei*. **UNITED KINGDOM:** Cornwall, 27 Apr 1954, Major Johnstone (KEW).

On *R. suavis*. **GERMANY?:** May 1894, G. v. Lagerheim (PUR F534).

On *R. usuriensis*. **CHINA:** Ji-dong-xian, Heilongjiang, L-P. Shao et al. (CFB 21586).

On *R.* sp. **NEW ZEALAND:** Stratford, Taranaki, 14 Apr 1924, J.C. Neill (K, Fungi of N.Z. 1714).

On *Picea abies*. **AUSTRIA:** Tirol, Brixen, 29 Jul 1891, P. Dietel (PUR F527). **ITALY:** Nr. Volga R., Piedmont, 1863, A. Carestia (PUR F513); Gressonay St. Jean, 1889, G. Briosi & F. Cavara (PUR F526). **SWITZERLAND:** Tr. to Nair L., St. Moritz, Engadine, 18 Aug 1998, R. Berndt (CFB 22181); Canton of Valais, 17 Aug 1955, E. Mayor (DAVFP 11953); Gurnigel, 1883, E. Fischer (PUR F325).

Chrysomyxa roanensis (Arthur) Arthur, Man. Rusts U.S. Can., p. 35. 1962.

Fig. 2.21, A–J

≡*Melampsoropsis roanensis* Arthur, Bull. Torrey Bot. Club, 49: 190. 1922.

Type: On *Rhododendron punctatum* Andr., Mt. LeConte (6600 ft), Tennessee, 8 Jun 1921, H.F. Bain, comm. J.A. Stevenson (Holotype PUR 4883! Isotypes BPI!).

Hosts and distribution: *Chrysomyxa roanensis* is known only from Tennessee and North Carolina on mountain summits above 1800 m, on *Rhododendron catawbiense* Michx., *R. minus* Michx., and *R. punctatum* (Anonymous 1960; Arthur 1934). Reports of occurrences elsewhere in North America in Farr et al. (1996), supposedly based on Arthur (1922), are in error. Similarly, Anderson's report (1952) of *C. roanensis* from northern British Columbia on *R. lapponicum* is likely a misidentification of *C. rhododendri*. A

sample examined from the same general area has urediniospores typical of the latter species. The aecial state of a needle rust on *Picea rubens* collected in the type locality likely belongs to *C. roanensis*.

Description: On *Rhododendron*. *Uredinia and telia* hypophyllous, on discolored spots. *Uredinia* in circular or elongated groups, 1/4–1 mm wide, cupulate, circular or irregular, subepidermal, with a peridium of large thin-walled cells. *Urediniospores* ellipsoidal or lenticular, 26–50(–56) × (15–)18–30 μm; wall hyaline, very thin, <0.5 μm; warts readily dehiscent, multiannulate, cylindrical to irregular and joined laterally, with poorly developed stiltlike bases, tops uneven or with a central depression; *wall + warts* 1.6–4.1 μm thick. *Telia* irregular, confluent in groups 2–3 mm wide, rusty-orange, surrounded by remnants of epidermis, appearing waxy when young. *Teliospores* catenulate, cuboid or subglobose, separating easily, 14–20 × 12–20 μm, wall 1 μm thick.

On *Picea* (unconfirmed). *Spermogonia and aecia* on current-year needles. *Spermogonia* amphigenous, subhypodermal, reddish brown; in cross section, triangular, with a well-defined, flat hymenium, 150–210 μm wide × 82–120 μm high. *Spermatia* globose to subglobose or pyriform, 3.3–4.5 × 2–4 μm. *Aecia* amphigenous. *Aeciospores* fusiform or ovoid, 32–52 × 16–23 μm, ends pointed or truncate where tops of warts join, part of a broad, well-defined longitudinal cap with a fissured edge and a surface of crowded shallow bumps that covers one-third to one-half of the spore; wall hyaline, 0.8 μm thick; warts as for urediniospores; *wall + warts* 2.8–3.3 μm thick. *Peridium* tubular, persistent, brownish at apex, rupturing mostly at apex; cells elongate, polygonal, narrow, and delicate, outside surface of cells shallowly concave, ± smooth, inside surface flat to shallowly concave with a raised edge, covered unevenly with shallow scattered warts.

Notes: The almost identical spore morphology of the urediniospores collected on *Rhododendron* and the aeciospores from adjacent *P. rubens* leaves little doubt that these are of the same species (Fig. 2.21, C–H). Inoculation experiments, however, are needed as confirmation and to provide more information on the life cycle of this localized and little-known rust.

Of all the North American species of *Chrysomyxa*, *C. roanensis* is undoubtedly most closely related to *C. piperiana*, based on its occurrence on *Rhododendron* and on the spore morphology. The main difference is that *C. piperiana* has longer narrower spores and slightly finer, narrower warts. The caplike longitudinal cover on one side of the aeciospores and urediniospores of *C. piperiana* also has a smoother appearance than in *C. roanensis* (Figs. 2.21, D; 2.15, E). Both have tubular aecial peridia with brownish cells at the apex, where dehiscence occurs. The shallow ornamentation of the peridial cells of both species is also remarkably similar, but warts are more distinct and well-defined in *C. roanensis* (Figs. 2.16, F; 2.21, J). Peridial cells of both species lack the striate side walls that are characteristic of nearly all other species examined. Interestingly, the spermatia of both species are also unusually large. Examination of more collections of both species might obscure these minor differences. A note by W.G. Ziller on one collection of *C. piperiana* on *R. macrophyllum* from Oregon (DAVFP 14507) supports this possibility: "Some uredinia have ellipsoid to pyriform spores as described for *C. roanensis*."

In *C. roanensis*, the broad longitudinal caplike cover over part of the urediniospores and aeciospores is also reminiscent of *C. empetri*. However, the warts of *C. roanensis*, in both the cap and elsewhere, are much coarser and longer than those of *C. empetri*. They are also less crowded and lack the complex basal connections characteristic of the warts of *C. empetri* (Figs. 2.6, 2.21). Compared with *C. rhododendri*, the spores of *C. roanensis* are much larger and their ornamentation much coarser.

Specimens examined: On *Picea rubens* (unconfirmed as aecial host). **USA: Tennessee:** Mt. LeConte (6600 ft), Great Smoky Mountains Natl. Park, 21 Aug 1939, J.A. Stevenson (BPI 140952); Mt. Le Conte, Myrtle Pt., 24 Sep 1935, H.M. Jennison (BPI 140953).

On *Rhododendron punctatum*. **USA: Tennessee:** Mt. LeConte (6600 ft), 8 Jun 1921, H.F. Bain, comm. J.A. Stevenson (PUR 4883, Type; BPI 140955, 180973, Isotypes); The Jumpoff, Mt. Kephart, Jun 1933, W.W. Diehl (BPI 140957); Alum Cave & Mt. LeConte (Myrtle Pt.), 21 Aug 1939, J.A. Stevenson (BPI 140970).

Chrysomyxa vaccinii (Ziller) P.E. Crane, comb. nov. **Figs. 2.22, A–C**

≡*Chrysomyxa ledi* var. *vaccinii* Ziller, in Savile, Can. J. Bot. 33: 492. 1955.

Type: On *Vaccinium parvifolium* Smith, Qualicum Beach, British Columbia, 17 Jun 1955, W.G.Z. (holotype DAOM 46500, isotypes DAVFP 9450! PUR 54765!).

Hosts and distribution: Telia and uredinia of *C. vaccinii* are known only on *Vaccinium parvifolium* Smith from Vancouver Island, Queen Charlotte Islands, and coastal British Columbia (Ziller 1974). Aecia are unknown, but likely occur on *P. sitchensis*, since telia occur regularly, and the rust has been collected near this spruce species.

Description: *Spermogonia and aecia* unknown.

On *Vaccinium parvifolium*. *Uredinia and telia* hypophyllous on overwintered leaves of previous year, on discolored patches between major veins. *Uredinia* in the same or different patches from telia, round to slightly elongated, 0.2–0.3 mm wide, subepidermal. *Urediniospores* variable in size and shape, mostly ellipsoidal, ovoid, or oblong, often with one or both ends flat, (16)20–32 × 11–20 μm; wall hyaline, 0.3–0.6 μm thick; *wall + warts* 1.6–2.5 μm thick; warts flat-topped, irregular in size and shape, annulate, stellate in surface view because of basal connections, sometimes joined into ridges. *Telia* in groups, single or confluent, sometimes in elongated ridges, subepidermal with flat hymenium, 0.2–0.7 mm wide, erumpent through epidermis. *Teliospores* catenulate, cuboid to oblong, 9–22 × 7–12 μm.

Notes: When Savile (1955) reduced several species of *Chrysomyxa* to varieties of *C. ledi*, he included Ziller's newly found species on *Vaccinium*, mainly because of spore size. However, one of the main reasons for establishing these varieties was the difficulty of identifying the aecial states on spruce. Because the aecial stage of *C. vaccinii* is so far unknown, inclusion of this species as a variety of *C. ledi* seems unjustified. It should also be noted that *C. vaccinii* has been reported to be most similar to *C. chiogenis* (Ziller 1974), which was not included in the *C. ledi* complex. SEM studies, along with hosts in subfamily Vaccinioideae, support the close relationship of these species (see Notes under *C. chiogenis*).

Chrysomyxa vaccinii is the only North American species in the genus that occurs on *Vaccinium*, perhaps because many species in this host genus have deciduous leaves and this would not permit overwintering of the mycelium. *Chrysomyxa taihaensis* Hiratsuka, f. & Hashioka occurs on *V. merrillianum* Hayata in Japan, but uredinia have not been reported for that species, and the teliospores are considerably larger (27–45 × 12–24 μm) than those of *C. vaccinii* (Hiratsuka and Hashioka 1935).

Inoculation of *Picea* and *Tsuga*, the two conifers found near the telial host (Savile 1955), with *C. vaccinii* basidiospores is needed to confirm the complete life cycle of this rust.

Specimens examined: On *Vaccinium parvifolium*. **CANADA: British Columbia:** Qualicum, Vancouver Is., 17 Jun 1955, W.G.Z. (PUR 54765, DAVFP 9450, Isotypes); Massett, Queen Charlotte Is., 8 Jul 1952, W.G.Z. (PUR 54528, ex DAOM 45774); Nr. Empire Anchorage, Athlow Bay, Graham Is., Queen Charlotte Is., 12 Jun 1957, J.A. Calder & D.B.O.S. (PUR 56660, ex DAOM 59520); Long Beach, Vancouver Is., 18 May 1968, W.G.Z. (DAVFP 18160).

C. weirii Jackson, Phytopathology, 7: 353. 1917. **Fig. 2.23, A–C**

Type: On *Picea engelmannii*, Whitman Natl. Forest, Oregon, 17 Jul 1913, J.R. Weir 271 (Holotype PUR 4907! Isotypes DAOM, DUKE, TRTC).

Hosts and distribution: *Chrysomyxa weirii* occurs sporadically in isolated, moist locations in boreal and subalpine forests (Weir 1923; Peterson 1961*b*) in northern Oregon, Idaho, western Montana, Washington, South Dakota, British Columbia, Alberta, Saskatchewan, Manitoba, New Brunswick, Northwest Territories, Yukon Territory, and the southern Appalachian Mountains on *P. engelmannii*, *P. glauca*, *P. mariana*, *P. rubens*, and *P. sitchensis* (Arthur 1934; Savile 1950; Ziller 1974). In recent years it has also been introduced to blue spruce (*P. pungens*) nurseries in the northeastern United States (Vermont, New Hampshire, Pennsylvania) (Pawuk 1971; Bergdahl and Smeltzer 1983; Merrill et al. 1993). It has also been reported in south-central Asia (Kuprevich and Tranzschel 1957).

Description: *Spermogonia*, *aecia* and *uredinia* unknown. *Telia* mandarin-orange (6B8), waxy and tonguelike when dry, on well-defined chlorotic bands on needles of previous year, 1/3–2 mm long, amphigenous, seldom confluent, dehiscent by longitudinal slit of host epidermis. *Teliospores* catenulate, 13–36(–42) × 5–9 μm, with one or two hyaline projections, 1.5–3.5 μm long at proximal end, and often a small knob at the distal end that will expand to form the basidium; variable in shape, irregular to ellipsoidal, cylindrical, or rhomboidal with ends obtuse, attenuate, or truncate; wall hyaline, smooth, 0.4–0.8 μm thick; spores extruded between flaps of the host epidermis, separating and dispersing readily when wet. *Basidia* cylindrical, two-celled, producing one basidiospore from each cell on a short sterigma. *Basidiospores* globose to subglobose, 5–6 × 5–6 μm, with a small apiculus.

Notes: This is the only autoecious microcyclic species of *Chrysomyxa* known to occur in North America. Weir (1923) confirmed its autoecious nature experimentally. Although it superficially resembles the European autoecious species *C. abietis*, the two taxa differ in a number of ways. The teliospores of *C. abietis* are wider (6–14 μm), and only the distal cells of the chains of cells in the telium are considered teliospores. The branching basal cells are sterile and do not germinate to produce basidia and basidiospores. *Chrysomyxa abietis* produces a four-celled basidium (Fig. 2.2, C) and four basidiospores within the sorus (Lindfors 1924; Grill et al. 1980; Hama 1987), whereas *C. weirii* teliospores are usually dispersed before they germinate (Fig. 2.23, B, C), and they form a two-celled basidium (Fig. 2.23, C). The basidial cells may also disarticulate to form two sporelike cells, or the basidium itself may function as a germ tube (Crane et al. 2000*a*). Morphological comparisons of this rust with microcyclic species endemic to Asia (*C. deformans* (Diet.) Jaczew., *C. tsugae-yunnanensis* Teng, *C. tsugae* Teng, *C. keteleeriae* (Tai) Wang & Peterson) have not been done. The two-celled basidium, though, appears to be unique to *C. weirii*. *Chrysomyxa deformans* is reported to have teliospores that separate at maturity (Kuprevich and Tranzschel 1957), but it is not known whether they function as diaspores as in *C. weirii*.

Chrysomyxa weirii overwinters as mycelium in needles of the previous year, and telia appear (Fig. 2.23, A) for a brief period in early spring the year after they have become infected. Young expanding current-year needles become infected after teliospores are dispersed onto their surface, likely by rain-splash or dripping water from foliage above (Crane et al. 2000*a*). Yellow banding of the needles is visible soon after infection (Ziller 1974).

Serious outbreaks in blue spruce nurseries have been reported in the northeastern United States in recent years (Pawuk 1971; Bergdahl and Smeltzer 1983; Merrill et al. 1993), and local occurrences of extensive defoliation sometimes occur in the Rocky Mountains (Can. For. Serv. 1963, 1968, 1970).

Specimens examined: On *P. engelmannii.* **CANADA: Alberta:** Marmot Cr. watershed, 20 mi SW of Seebe, 12 Jul 1967, G.J. Smith (CFB 7967). **British Columbia:** Vermilion Crossing, Kootenay Natl. Park, 12 Jun 1953, J.K. Robins (CFB 458); SE slope Mt. Wardle, 6 mi NE Kootenay Warden Stn., 20 Jun 1960, G.J. Smith (CFB 7960); mi 2.4 Bear Cr. Rd., Squilax, 27 May 1969, D. Doidge (CFB 22111, ex DAVFP 69-9-0775-01). **USA: Oregon:** Whitman Natl. For., 17 Jul 1913, J.R. Weir (PUR 4907, Type). **Washington:** Bear Cr. Camp, Umatilla Natl. For., Garfield Co., 7 Jun 1949, Shaw et al. (PUR 59389).

On *P. glauca.* **CANADA: Alberta:** Graburn Cabin, Elkwater, 4 Jun 1962, E. Gautreau (CFB 4903); 100 mi N of Peace River, 24 Jun 1963, N.W. Wilkinson & E. Gautreau (CFB 5742); Junction of east boundary of Range 5 and Clearwater R., 28 Jun 1967, C. Layton (CFB 8089); 15 mi E of Clearwater L., Cypress Hills Prov. Park, 10 Jun 1970 (CFB 20015); Red Earth Cr., Banff Natl. Park, 5 Jul 1997, P.E & S. Crane (CFB 22077); Miette Hot Springs, Jasper Natl. Park, 22 Jun 1999, P.E. & A. Crane (CFB 22195, 22196); Ribbon Cr. Trail, Kananaskis, 1 Jun 1998, P.E.C. (CFB 22198). **British Columbia:** 4 mi SW of Field, nr. mouth of Amiskwi R., Yoho Natl. Park, 23 Jun 1968, G.J. Smith (CFB 8498). **Yukon:** Mi 674, Alaska Hwy, 24 Jun 1966, R.W. Barry (CFB 7495); mi 29.7, Cantung Rd., Watson L., 24 Jun 1969 (CFB 8904). **Saskatchewan:** Jct. Trail 57 & Narrows Rd., Prince Albert Natl. Park, 20 Jun 1972, E. Gautreau (CFB 20816).

On *P. mariana.* **CANADA: Manitoba:** Duck Mtn. Prov. Park, 5 Jun 1998, M. Michaelian (CFB 22175).

On *P. pungens*. **USA: New York:** Philwood Estates, St. Joseph's, Sullivan Co., J.B. Harry (PUR 49269).

On *P. rubens*. **USA: West Virginia:** Black Mtn, Pocahontas Co., 4500 ft, 14 Jun 1933, C.R. Orton (PUR 44396).

On *P. sitchensis*. **USA: Oregon:** Government Camp, 6 Jun 1969, Y.H. & P.J. Maruyama (CFB 8818).

Chrysomyxa woroninii Tranz., Centralbl. Bakteriol., Parasitenk. Abt. 2, 11: 106. 1903.

Fig. 2.24, A–H

=*Aecidium coruscans* Fr., Physiogr. Sällskap. Aarsb., Lund, p. 92. 1824.

=*Peridermium coruscans* Fr., Summ. Veg. Scand., p. 510. 1849.

Type: On *Ledum palustre*, Levashovo, near Leningrad, Russia, 1903, W. Tranzschel (Isotype PUR F510!).

Hosts and distribution: *Chrysomyxa woroninii* probably has a circumboreal distribution, but is restricted to far northern or subalpine areas where the two hosts occur together. It has been reported in northern Canada and Alaska (Savile 1950, 1955; McBeath 1981, 1984), northern Europe (Scandinavia, Finland, Russia) (Tranzschel 1903; Jørstad 1937; Hylander et al. 1953), Siberia and Kamchatka (Azbukina 1974; Jørstad 1934), northeastern China (He et al. 1995), and southwestern China (Liu and Bau 1980; Kuprevich and Tranzschel 1957). Reports by Spaulding (1961) of the rust in India, Japan, and Britain are unconfirmed. Telial hosts are *Ledum decumbens* and *L. groenlandicum* in North America, *L. palustre* in Eurasia, and *L. hypoleucum* Kom. and *L. decumbens* in the Russian Far East. Spruce species known to be affected are *Picea glauca*, *P. mariana*, *P. abies*, *P. pungens*, *P. koraiensis*, *P. ajanensis*, *P. obovata*, and *P. likiangensis*.

Description: On *Ledum*. Systemic and perennial in shoots of witches' brooms. *Uredinia* absent. *Telia* subepidermal, deep orange (5A8) to brownish orange (6C8), forming almost continuous crusts on underside of current-year leaves. *Teliospores* catenulate, thin-walled, cuboid when young, becoming more rounded and oblong or

subglobose at maturity. *Basidia* slightly curved, 4-celled, with broad sterigmata. *Basidiospores* globose to subglobose or pyriform, with a small pointed or truncate apiculus, 6–11 × 6–10 μm.

On *Picea*. *Spermogonia and aecia* on stunted current-year needles of systemically infected vegetative buds or ovulate strobili. *Spermogonia* amphigenous, at tips of needles, pale, becoming reddish brown; in cross section ± globose, 90–180 μm wide × 80–140 μm high, hymenium concave. *Spermatia* ellipsoidal, ~4 × 1.3 μm. *Aecia* amphigenous, almost the full length of the needle, erumpent through the epidermis; in cross section, shallow and cupulate. *Aeciospores* extremely variable in size and shape, ovoid, ellipsoidal, fusiform, or pyriform, occasionally subglobose or globose, sometimes slightly flattened at ends or with a narrow tail, 24–50 or longer × (12)17–35 μm, light orange (5A5); wall hyaline, distinct from warts, 1.2–2.5 μm thick; warts 1.2–3.3 μm high, polygonal in surface view, truncate or irregular at apex, may be confluent at spore ends; *wall + warts* 2.5–5.7 μm thick. *Peridium* opening irregularly at maturity, composed of rounded or elongated overlapping cells; inner surface of cells shallowly concave, very finely and densely warted; outer surface concave, rugose.

Notes: Tranzschel (1903) first suggested the connection of *C. woroninii* with *Peridermium coruscans* Fr. based on the constant field association of the telia on *Ledum palustre* and the aecia on *Picea abies*. However, the life cycle has remained controversial until recently because experimental proof was lacking and other species of *Chrysomyxa* are often present on the same hosts. Field observations and successful inoculation of *P. mariana* with basidiospores from *L. groenlandicum* have finally proven the connection and confirmed other aspects of the life cycle (Crane et al. 2000*b*). The life cycle requires 2 years to complete, unlike nonsystemic spruce needle rusts. Infection of spruce by basidiospores occurs during spring of the first year, likely through young needles, and mycelium grows into shoots, where it remains until the next spring. Production of spermogonia and aecia occurs on needles of the newly opened buds, which are systemically infected (Fig. 2.24, B, C). The fungus can survive in the *Ledum* host perennially, but in spruce the infected buds die after sporulation. The rust does not kill its

Ledum hosts, but usually causes brooming on just part of the plant (Fig. 2.24, A). Young leaves die and are shed after telia are produced, but the brooms produce other leaves that appear relatively healthy, though stunted. *Chrysomyxa ledicola*, *C. reticulata*, *C. nagodhii*, and *C. ledi* may occur on both broomed and healthy shoots of the same plants as *C. woroninii*, but in spring only on leaves of the previous year. Infected spruce buds and infected *Ledum* are usually found in close proximity in the field (Fig. 2.24, A).

Heavy infection (up to 70% of new shoots) of young spruce by *C. woroninii* has been observed in Alaska. Such heavy infection may retard growth, and infection of female cones may reduce spruce regeneration near treeline (McBeath 1981, 1984).

The relationship of *C. woroninii* to Asian species of *Chrysomyxa* causing systemic shoot infections in spruce and witches' brooms on rhododendrons needs examination. *Chrysomyxa komarovii* Tranz. was separated from *C. woroninii* because it is found on *Rhododendron dauricum* L. rather than *Ledum* (Kuprevich and Tranzschel 1957). However, this may not be a valid criterion for separating the two species. As noted elsewhere, *Ledum* is now considered a subgenus of *Rhododendron*, and the same rusts often affect members of both (see descriptions of *C. nagodhii*, *C. reticulata*, and *C. ledicola*). *Peridermium thomsoni* Berk., found in India on *Picea smithiana*, also causes systemic shoot infections (Bakshi and Singh 1967). Further study is needed to determine the relationship of these taxa.

Specimens examined: On *Ledum decumbens*. **CANADA: British Columbia:** Mi 402, Alaska Hwy., Summit L., 15 Jun 1972, C.S. Wood (CFB 22095, ex DAVFP 20264).

On *L. groenlandicum*. **CANADA: Alberta:** Little Berland R., 67 km NW of Hinton, 19 Jun 1997, Y.H. & P.E.C. (CFB 22121). **British Columbia:** Mi 28, Dease L. Rd., S of Cassiar, 25 Jun 1973, C.S. Wood (CFB 22114, ex DAVFP 20528).

On *L. palustre*. **RUSSIA:** Levashovo, 2 Jun 1903, W. Tranzschel (PUR F510, Type locality).

On *Picea abies*. **FINLAND:** Kivijärvi, Muonio, 24 Jun 1983, R. Jalkanen (CFB 22167); Ruosselkä, Vuotso, 6 Aug 1998, Y.H. & P.E.C. (CFB 22201). **SWEDEN:** Stockholm, 19 Jun 1884, O. Juel (PUR 520).

On *P. glauca*. **CANADA: Alberta:** 20 km SE of Grande Cache, 27 Jun 1995 (CFB 22014); Little Berland R., N of Hinton, Jun 1997, P.E.C. (CFB, no No., CFB 22036);

On *P. mariana*. **CANADA: Alberta;** Edmonton, 28 May 1998, P.E.C. (CFB 22172, from inoculation). **Yukon Territory:** 33 mi W. of Dawson, 15 Jul 1959, W.G.Z. (CFB 22116, ex DAVFP 11418).

A NEW PERIDERMIUM ON *Picea sitchensis*

Peridermium zilleri P.E. Crane, anam. nov. **Fig. 2.25, A–E**

Type: On *Picea sitchensis*, Yakoun L., Graham Is., Queen Charlotte Is., British Columbia, 12 Sep 1988, D. Redfern (Holotype DAVFP 23796, Isotype DAOM 198870).

Hosts and Distribution: *Peridermium zilleri* is known on *Picea sitchensis* from the Queen Charlotte Islands, coastal British Columbia, and from the southwest coast of Vancouver Island. Although a specimen from Vancouver Island had shorter spores than the samples from the Queen Charlotte Islands, the morphology was very similar and therefore it is included here.

Description: In *Picea sitchensis*. *Spermogonia* ignota. *Aecia* in lineis chloroticis acuum arborearum huius anni, in ordinibus duobus in superficie supera, discreta, angusta, similia tubis, 1/2–1 1/4 mm lata. *Aeciosporae* fusiformae, 23–29 (–44) × 10–19 μm, truncatae vel in extremis acutae; pars sporae velata a superficie lata et longitudinale et leviore de summis verrucis dorsa formantibus; alibi verrucae cylindricae et annulatae, bases grallae-formes; summae latae et irregulares aspectu, 1.6 μm altae; tunica indistincta, 0.8 μm vel magis tenuis. *Peridium* persistens, cum cellulis in apice brunneis, dehiscens in lateribus; cellulae elongatae, superficies externa levis, paulo concava; superficies interna paulo convexa, cum verrucis longis et angustis et frequenter congregatis, nonnumquam speciem labyrinthi formantibus.

Etymology: Named in honor of W.G. Ziller, an authority on tree rusts and former curator of the Mycological Herbarium, Pacific Forestry Centre, Canadian Forest Service, Victoria, British Columbia.

On *Picea sitchensis*. *Spermogonia* unknown. *Aecia* on chlorotic bands of current-year needles, in two rows on upper surface, discrete, narrow, tubelike, 1/2–1 1/4 mm wide. *Aeciospores* fusiform, 23–39(–44) × 10–19 μm, truncate or pointed at ends; part of spore covered with a broad longitudinal smoother area consisting of wart tops joined into ridges; warts elsewhere cylindrical and annulate, with stiltlike bases, tops broad and irregular in surface view, 1.6 μm high; wall indistinct, 0.8 μm or less thick. *Peridium* persistent, with brownish cells at apex, dehiscent at sides; cells elongated, outer surface smooth, shallowly concave; inner surface slightly convex, with long, narrow, crowded warts, sometimes joined into a mazelike pattern.

Notes: The similar aeciospore morphology of *P. zilleri* to species in the genus *Chrysomyxa* suggests that it belongs in that genus. However, without spermogonia, this anamorph cannot be placed with certainty in that genus, and may also belong in *Pucciniastrum*. Two species of *Chrysomyxa* also lack spermogonia: *C. chiogenis* and the west coast variety of *C. ledicola*. Although *P. zilleri* resembles *C. chiogenis* in morphology, the only reported host of *C. chiogenis*, *Gaultheria hispidula*, does not occur on either of the islands where this *Peridermium* is found (Pojar and Mackinnon 1994; Clark 1998; Douglas et al. 1999). Aeciospores of *P. zilleri* differ from those of *C. chiogenis* in their longer, narrower shape; more obvious caplike ends; and less developed basal connections between warts. Both have a broad longitudinal smoother area on the aeciospores, but in *P. zilleri* it is not continuous, but broken into ridges (Fig. 2.25, A–C). The inner surface of the peridial cells of *C. chiogenis* is shallowly and sparsely ornamented, whereas in *P. zilleri* the warts are long, narrow, and densely crowded. Spore morphology and size are also different from the other west coast species with elongated spores, *C. piperiana*. For the above reasons, it seems desirable to recognize this rust as a distinct species.

Based on distribution and spore morphology, the most likely telial state of *P. zilleri* is *C. vaccinii*, for which inoculation experiments have not been done to determine the aecial state.

Specimens examined: On *Picea sitchensis*. **CANADA: British Columbia:** Queen Charlotte Islands: Port Clements, 27 Aug 1943, R.E. Foster (DAVFP 328, with *C. ledicola*); Alliford Bay, 12 Sep 1988, D. Redfern (DAVFP 23794, ex DAOM 198869); Yakoun L., Graham Is., 12 Sep. 1988, D. Redfern (DAVFP 23796, ex DAOM 198870). Vancouver Is.: Port Renfrew, 15 Nov 1972, G.W. Coombs (DAVFP 20419).

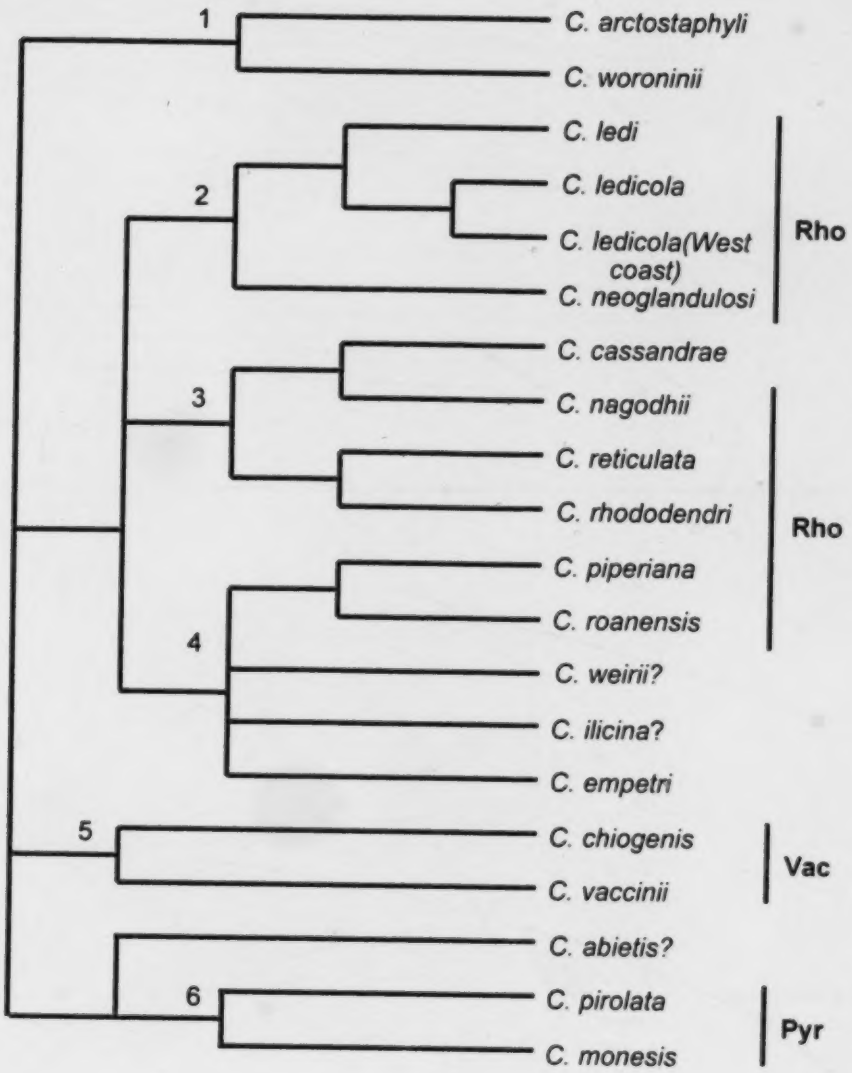
1
C. arctostaphyli
C. woroninii
C. ledi
C. ledicola
2
C. ledicola(West coast)
C. neoglandulosi
Rho
C. cassandrae
C. nagodhii
3
C. reticulata
C. rhododendri
Rho
C. piperiana
C. roanensis
4
C. weirii?
C. ilicina?
C. empetri
5
C. chiogenis
C. vaccinii
Vac
C. abietis?
6
C. pirolata
C. monesis
Pyr

Fig. 2.2. *Chrysomyxa abietis*. (A) Cross section of telium, showing slightly constricted base consisting of chains of sterile basal cells. (B) Two teliospores arising from a collapsed sterile basal cell (arrow). (C) Teliospores germinating *in situ* to produce four-celled basidia. Bars in A–C = 20 μm.

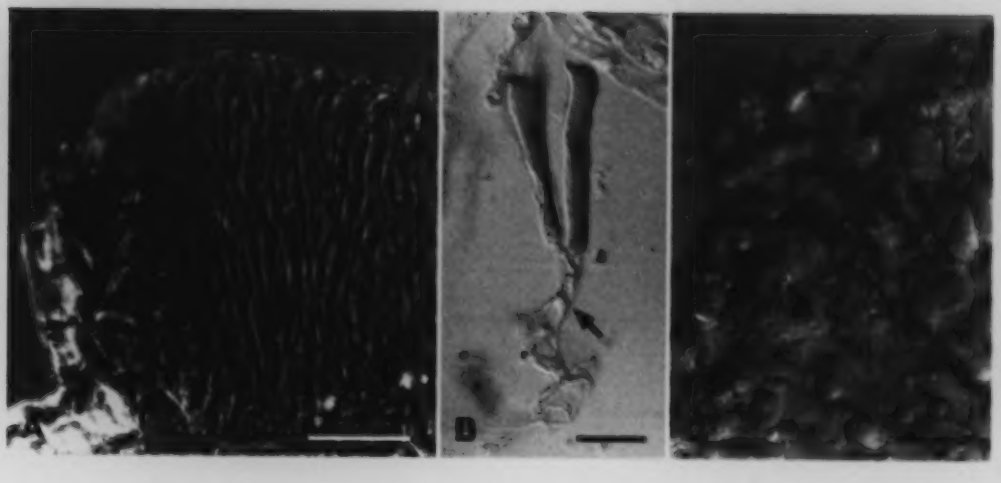
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Fig. 2.3. *Chrysomyxa arctostaphyli.* (A) Groups of hypophyllous telia (arrows) on discolored leaf spots of *Arctostaphylos uva-ursi.* (B) Upright perennial witches' broom (arrow) on a spruce branch. (C) Systemically infected spruce needles covered with spermogonia (arrow) and aecia in early summer. (D) Cross section of spruce needle with globose, subepidermal spermogonium. (E) Light micrograph of aeciospores. (F-I) Scanning electron micrographs of (F) aeciospore, (G) aeciospore surface ornamentation, (H) outer aecial peridium, and (I) inner aecial peridium. Bars in A, C = 2 mm; in B = 4 cm; in D = 40 μm; in E = 15 μm; in H, I = 20 μm.

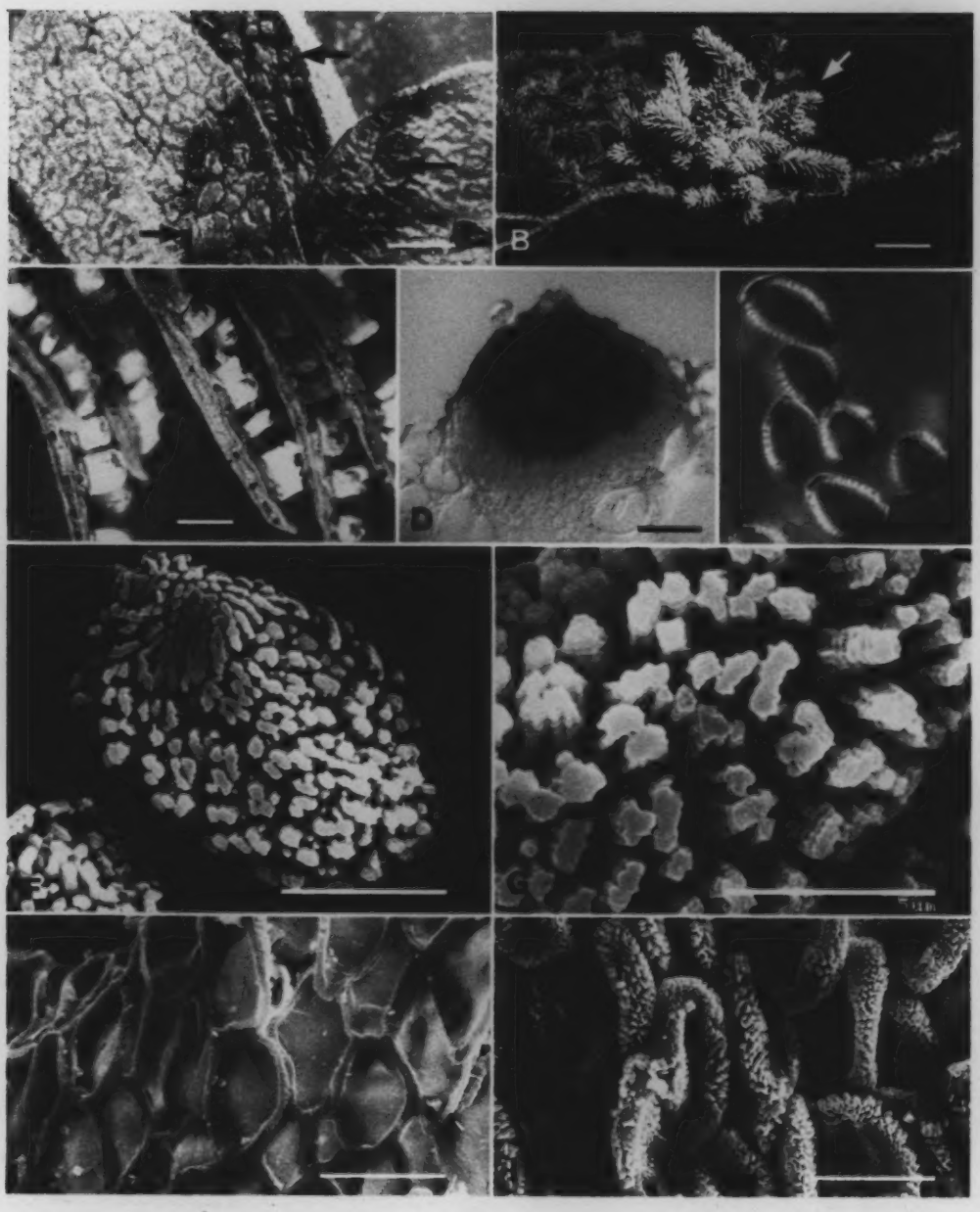
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Fig. 2.4. *Chrysomyxa cassandrae*. (A) Leaf of *Chamaedaphne calyculata* with hypophyllous telial crusts (CFB 22069). (B) Leaf of *C. calyculata* with uredinia on midvein and petiole. Remnants of peridium are visible (arrow). (C) Heavily infected blue spruce (*Picea pungens*) from artificial inoculation (CFB 22126). (D–F) Urediniospores: (D) by LM, (E) single spore (SEM) showing broad longitudinal cap; (F) details of ornamentation. (G–I) Aeciospores: (G) by LM, (H) single spore (SEM) , showing cap with bumps and broken edge; (I) details of ornamentation. (J, K) Aecial peridium: outer surface (J) and inner surface (K). Bar in A = 2 mm; in B = 1.5 mm; in D, E, G, H = 10 μm; in F, I = 2 μm; in J, K = 20 μm.

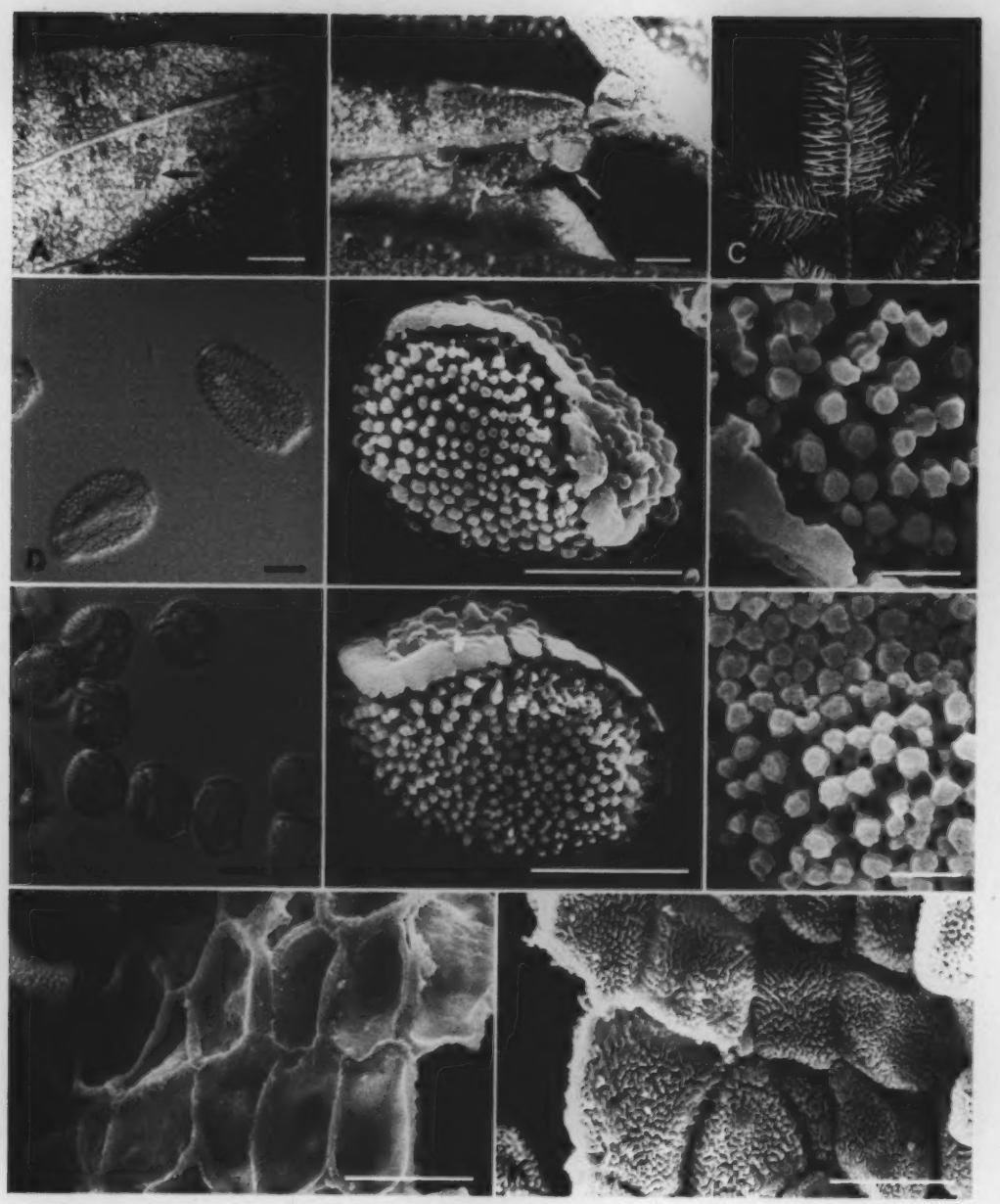
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Fig. 2.5. *Chrysomyxa chiogenis*. (A) Leaf (about 1 cm long) of *Gaultheria hispidula* with scattered uredinia and telia on abaxial surface (Type). (B–E) Urediniospores: (B) LM, Prince George, B.C.; (C) SEM of single spore, Quebec; (D) SEM of single spore showing warts joined into ridges, British Columbia, and (E) surface ornamentation, showing bumpy, pitted warts. (F–H) Aeciospores (from inoculation, BPI 190499): (F) LM, (G) SEM of single spore, and (H) surface ornamentation. (I, J) Aecial peridium: (I) smooth outer surface and (J) warted inner surface. Bars in B, C, D, F, G = 10 μm; in E and H = 2 μm; in I and J = 20 μm.

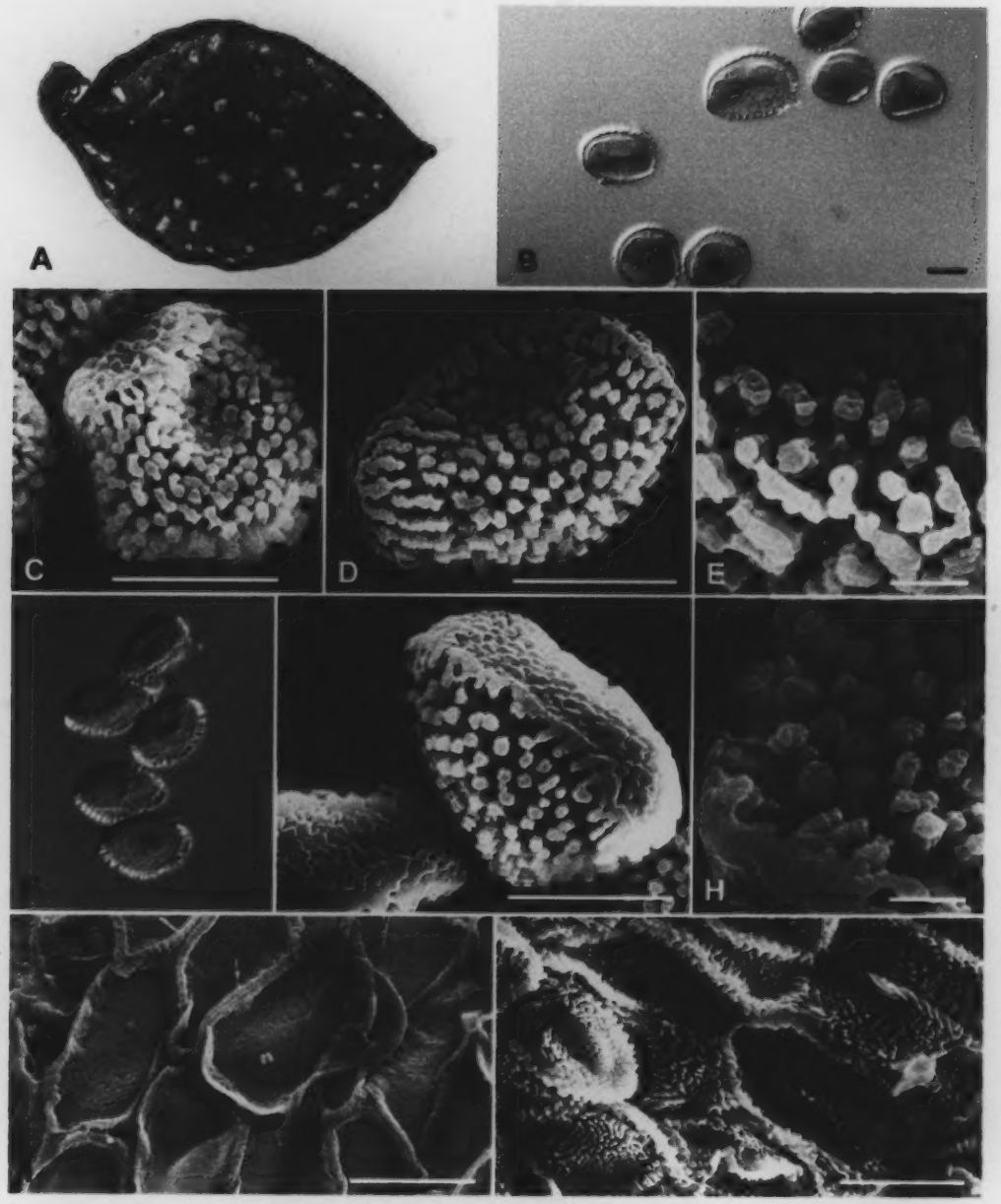
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Fig. 2.6. *Chrysomyxa empetri.* (A) Shoot of *Empetrum nigum* with uredinia (arrows) on abaxial surface of leaves from previous year, Alberta. (B) Leaves of *E. nigrum* bearing velvety telia (top) and dehiscent uredinia (bottom), releasing urediniospores (PUR 47904, Quebec). (C–E) Urediniospores: (C) by LM, (D) SEM of single spore, showing broad punctate longitudinal cap, Russia (TSH R9256), and (E) small warts with complex basal connections. (F–H) Aeciospores: (F) by LM, (G) SEM of single spore, (H) surface ornamentation. (I, J) Aecial peridium: (I) deeply concave, punctate outer surface, and (J) densely warted inner surface. Bar in A = 1 mm; in B = 1.5 mm; in C, D, F, G = 10 μm; in E = 2 μm; in H = 5 μm.

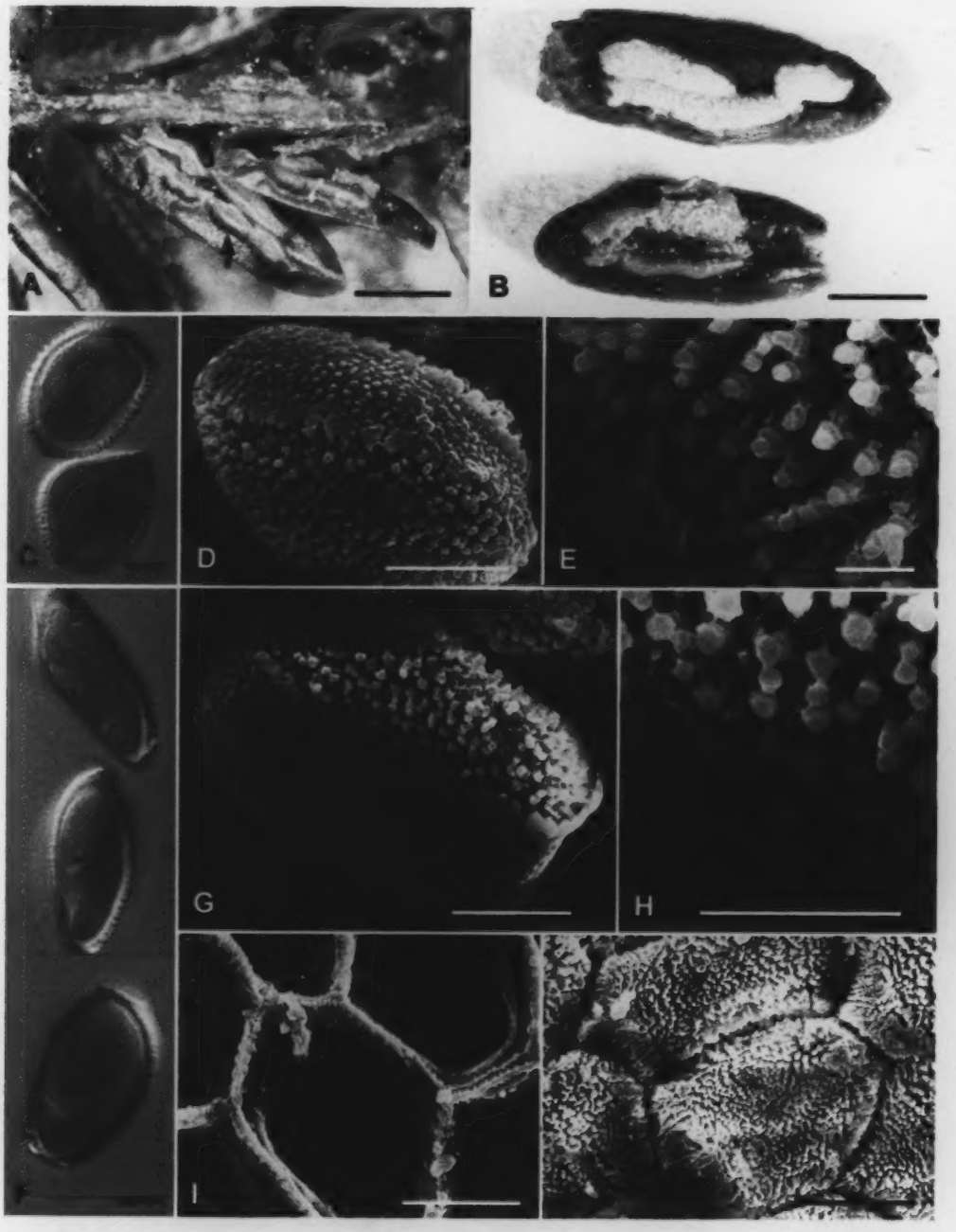
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Fig. 2.7. *Chrysomyxa ilicina.* (A) Leaf of *Ilex opaca* with groups of uredinia on abaxial surface; (B) uredinia and released urediniospores on leaf (PUR 44345, Tennessee). (C, D) Cross sections of telia, showing two-celled teliospores (arrowheads) and a chain of ornamented urediniospores (D, arrow) forming within a telium (PUR 44346, Tennessee). (E–G) Urediniospores (BPI 190526, Texas): (E) by LM, (F) single urediniospore by SEM, and (G) surface ornamentation. Bar in A = about 5 mm; in B = about 1 mm; in C–F = 10 μm; in G = 5 μm.

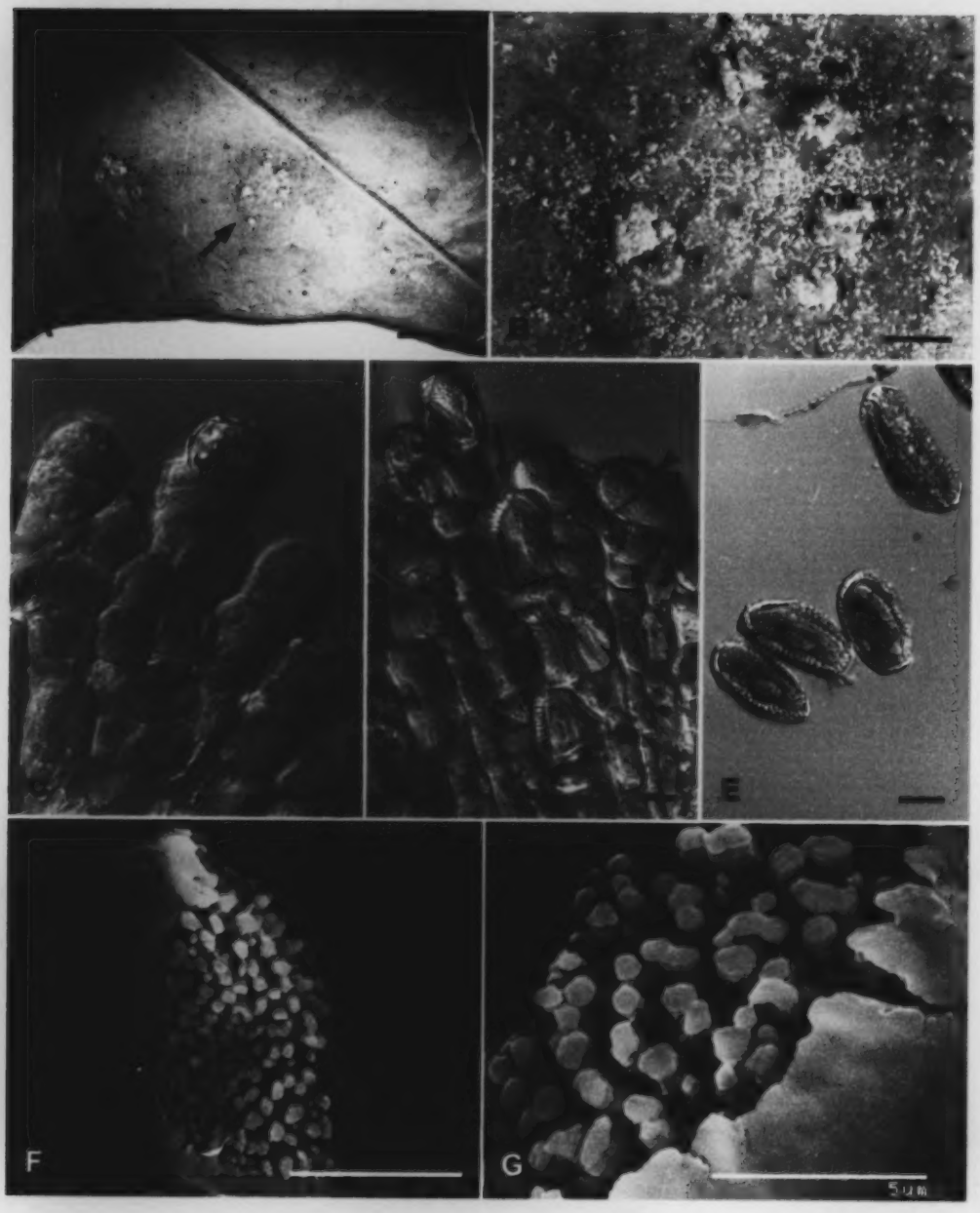
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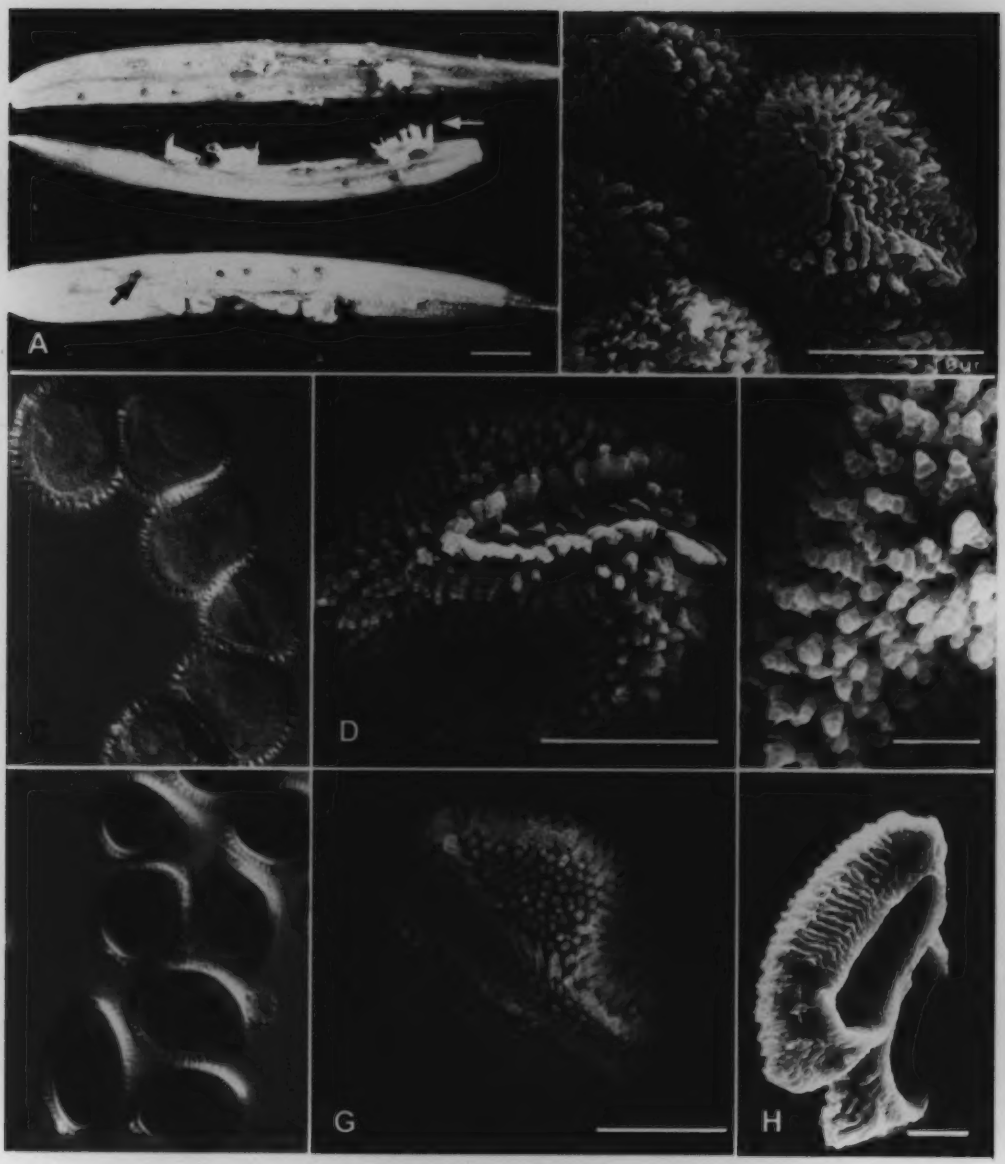
Fig. 2.8. *Chrysomyxa ledi*. (A) Infected needles of *Picea abies* with spermogonia (black arrow) and aecia (white arrow). Note shredding of aecial peridium. (B) SEM of urediniospores, Japan. (C–E) Urediniospores, Europe: (C) by LM, (D) single spore with well-defined groove, and (E) narrow, tapered warts. (F, G) Aeciospores. (H) Aecial peridium, showing broad, coarsely striate side walls and concave outer surface of cells. Bar in A = 1.5 mm; in B–D, F–H, = 10 μm; in E = 2 μm.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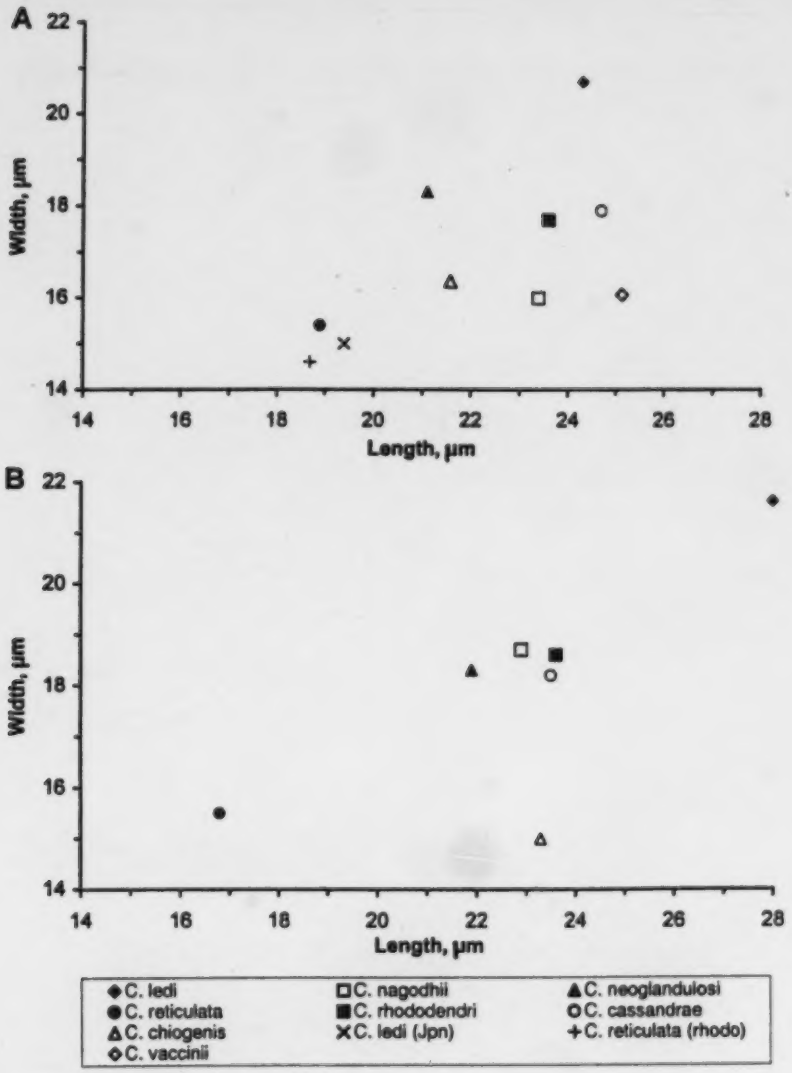

Fig. 2.9. Comparison of mean size of (A) urediniospores and (B) aeciospores of *C. reticulata*, *C. chiogenis*, *C. ledi s.s.* and species of *Chrysomyxa* formerly considered varieties of *C. ledi* (*C. cassandrae*, *C. neoglandulosi*, *C. nagodhii*, and *C. vaccinii*). Note much smaller size of *C. ledi* urediniospores from Japan compared with those from Europe; larger size of *C. ledi* compared with *Ledum* rusts from North America; and congruence of *C. reticulata* urediniospores on *Ledum* and *Rhododendron* ("*C. reticulata* (rhodo)"). Aeciospores of *C. ledi* from Japan and *C. vaccinii* are unknown.

Fig. 2.10. *Chrysomyxa ledicola* on *Ledum groenlandicum*. (A) Uredinia (short arrow) and telia (long arrow) on upper leaf surface. At the right, uredinia are forming within old telia. (B) Cross section of telium showing chains of thin-walled teliospores. (C) Four-celled basidia (arrows) forming from germinating teliospores. (D–J) Morphological variability in urediniospores from three locations in North America: (D, E) Tacoma, Washington (PUR 4777), from pods and peduncles; (F–H) Hinton, Alberta, from leaves (CFB 22011); (I, J) Mt. McIntyre, New York, from leaves. Bars in A = 1 mm; in B, C, F = 20 μm; in D, G, I = 10 μm; in E, H, J = 2 μm.

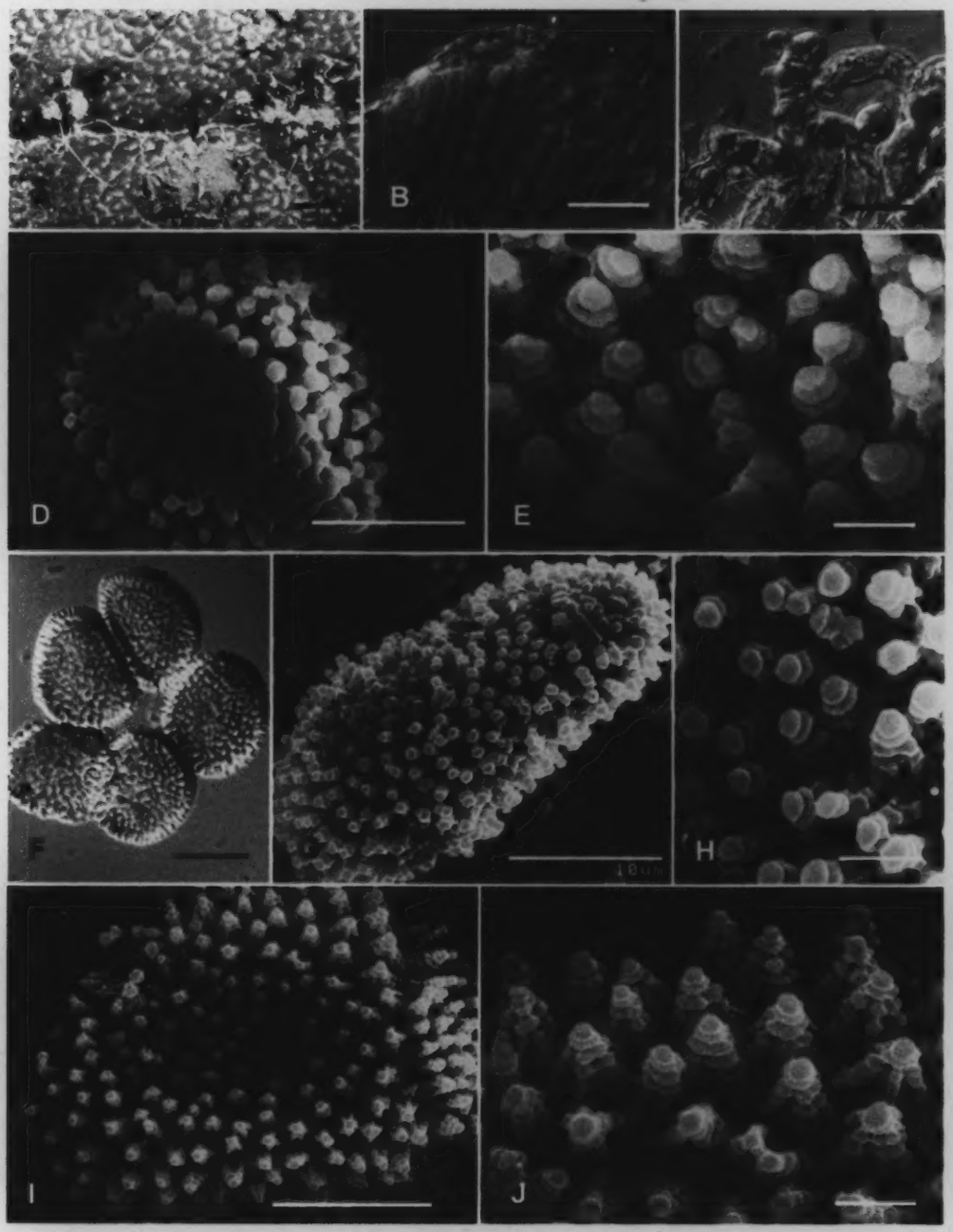
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Fig. 2.11. *Chrysomyxa ledicola* on *Picea* spp. (A) Needle with unopened aecia. (B–F) Morphological variability in aeciospores from three locations in North America: (B, F) New Hampshire (PUR 59403), (C) west-central Alberta (CFB 5743); (D, E) Prince Rupert, B.C. (CFB 22100, ex DAVFP 7018). (G) Aeciospores from central Alberta by LM. (H, I) Aecial peridium: (H) shallowly warted inner surface, (I) deeply concave outer surface, showing coarsely striate side-walls. Bars in A = 2 mm; in B, C, D = 10 μm; in E, F = 2 μm; in G–I = 20 μm.

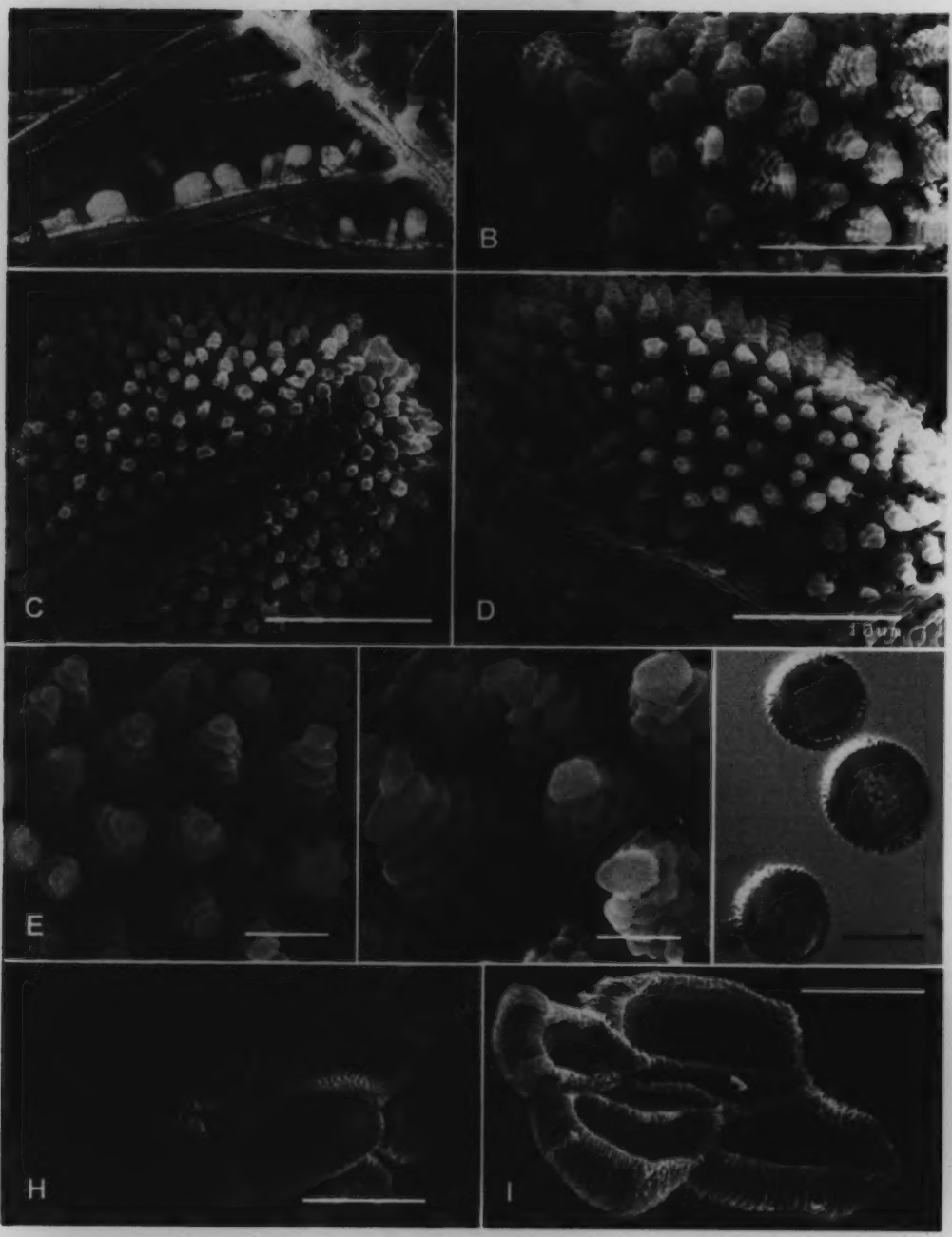
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Fig. 2.12. *Chrysomyxa monesis*. (A, B) Uredinia on *Moneses uniflora*: flower (A) and young leaf (B). Note conspicuous fringe (A, arrow) around uredinia formed by split host epidermis. (C–E) Urediniospores: (C) by LM, (D) single spore by SEM, and (E) details of ornamentation. (F–H) Aeciospores: (F) single spore by SEM, (G) details of ornamentation, and (H) by LM. (I) Single cell of evanescent aecial peridium. Bars in A, B = 4 mm; in C, H = 20 μm; in D, F, I = 10 μm; in E = 2 μm; in G = 5 μm.

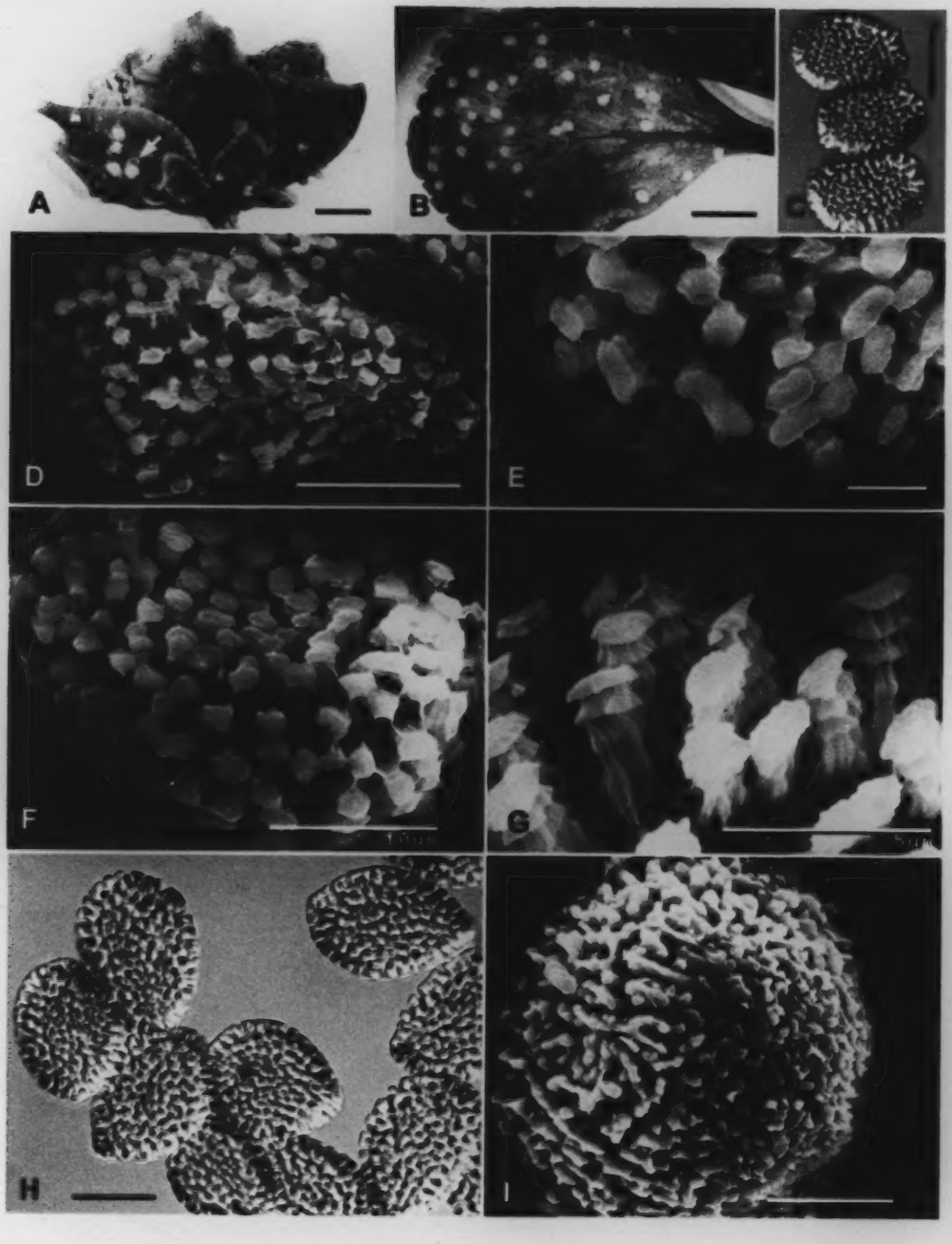
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Fig. 2.13. *Chrysomyxa nagodhii*. (A) Column of urediniospores extruded from uredinium on underside of *L. groenlandicum* leaf (SEM). (B) Cross section of uredinium. Note peridium of several layers of cells (arrow). (C) Cross section of telium. (D, E) Urediniospores by LM (D) and SEM (E), showing almost-smooth surface (central Alberta, CFB 22066). (F–H) Aeciospores: (F) by LM and (G) SEM, and (H) details of surface ornamentation (on *Picea pungens*, from inoculation, CFB 22186). (I, J) Aecial peridium: (I) outer surface and (J) inner surface. (K) A mite, *Dentizetes ledensis*, commonly found on infected *L. groenlandicum*, carrying urediniospores on the surface (central Alberta). Bars in A, K = 100 μm; in B–G = 10 μm; in H = 2 μm; in I, J = 20 μm.

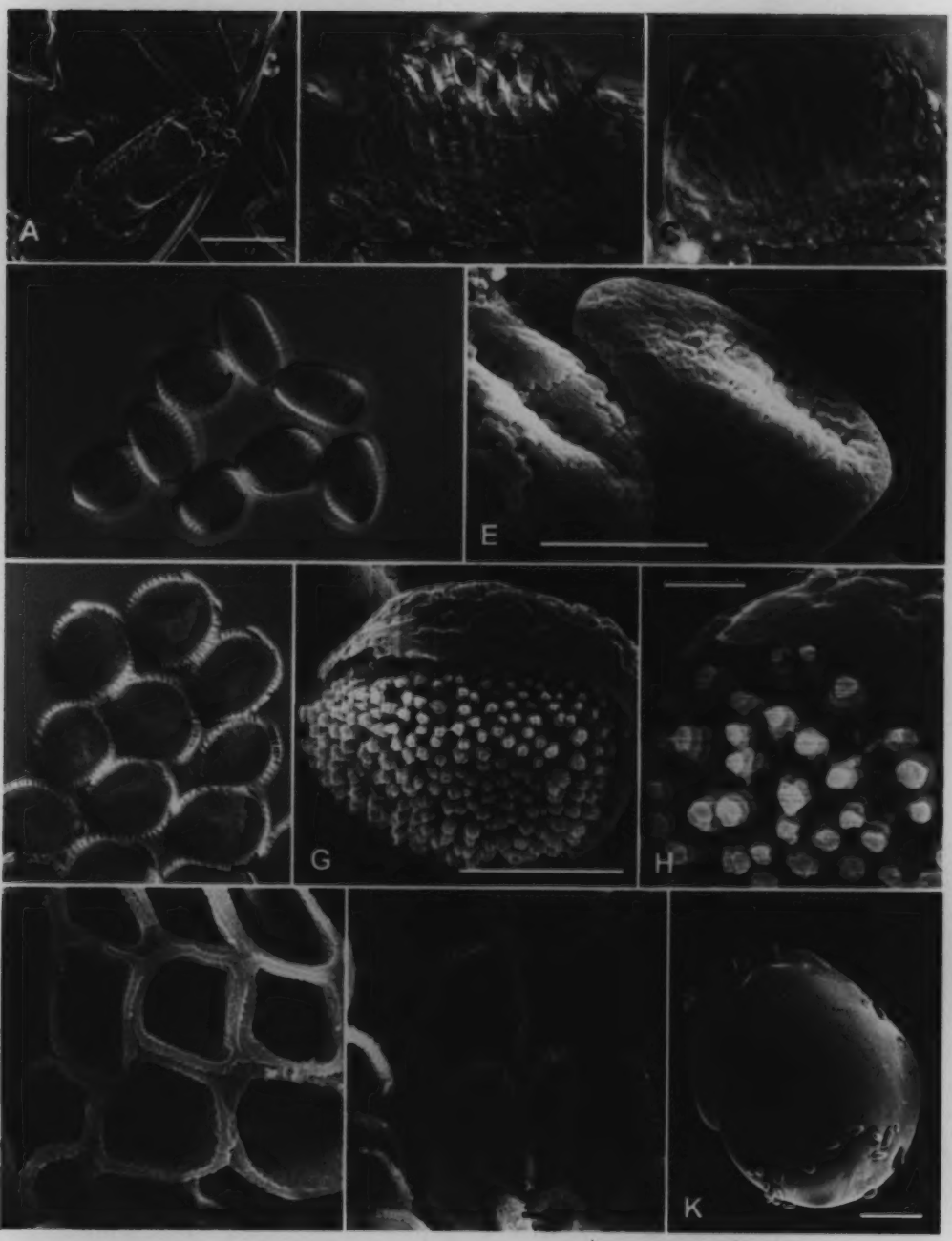
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Fig. 2.14. *Chrysomyxa neoglandulosi*. (A) Leaf spots (arrows) on infected *L. glandulosum* adjacent to *P. engelmannii*, type locality, Waterton, Alberta. (B) Cross section of leaf with uredinium. (C–E) Globose urediniospores: (C) by LM, (D) single spore (SEM), and (E) very fine, almost echinulate warts. (F–H) Aeciospores: (F) by LM, (G, H) showing very fine, narrow warts with annuli barely visible (SEM). (I, J) Aecial peridium: (I) outer surface and (J) inner surface. Note coarsely striate margins and dense ornamentation of outer surface. Bar in B = 100 μm; in C, D, F, G = 10 μm; in E, H = 2 μm; in I, J = 20 μm.

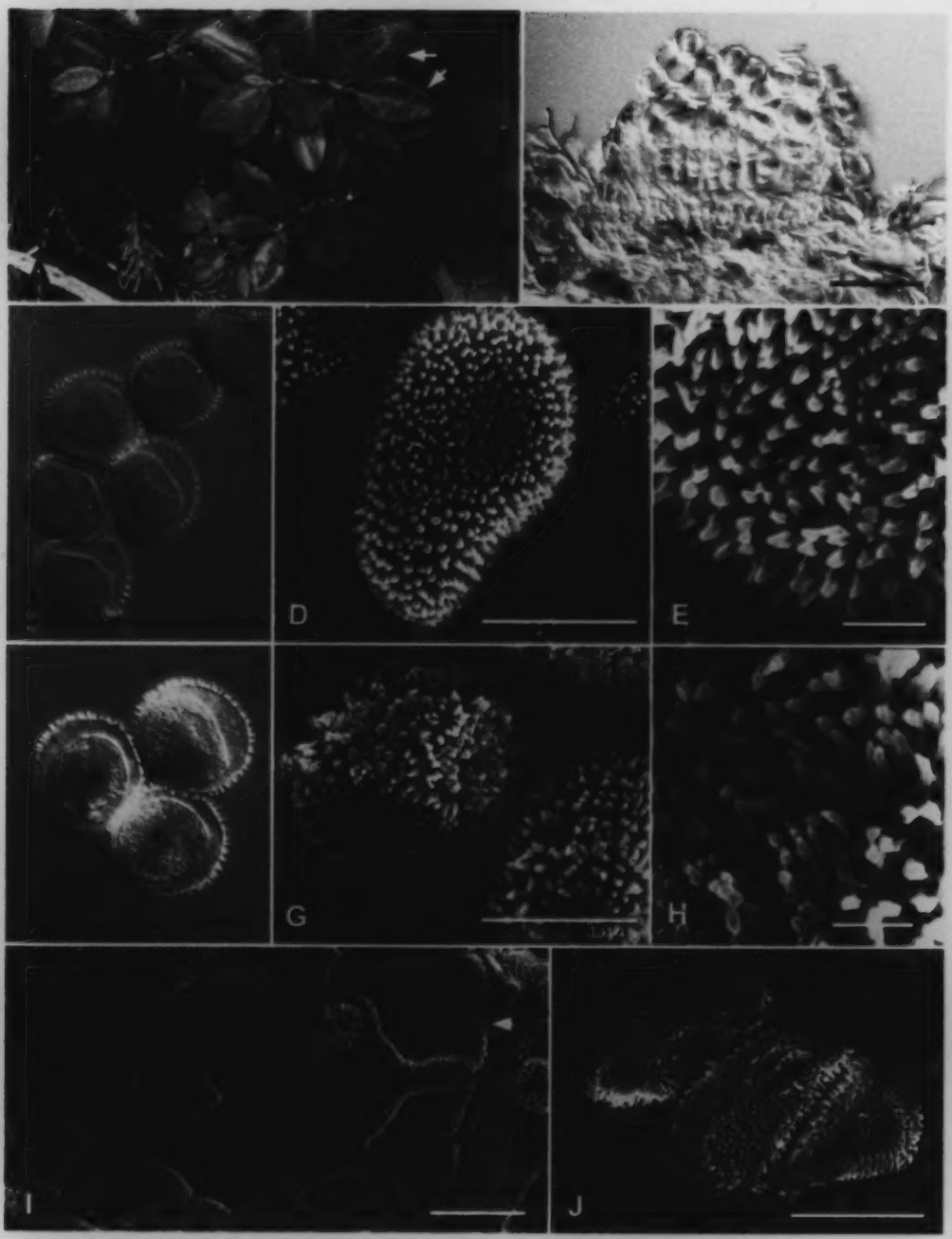
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Fig. 2.15. *Chrysomyxa piperiana* on *Rhododendron macrophyllum*. (A) Telia on underside of leaf. (B) Uredinia and urediniospores on underside of leaf. (C–F) Urediniospores: (C) by LM, (D) by SEM, (E) details of longitudinal cap, and (F) surface warts. Bars in A, B = 1 mm; in C, D = 20 μm; in E = 10 μm; in F = 2 μm.

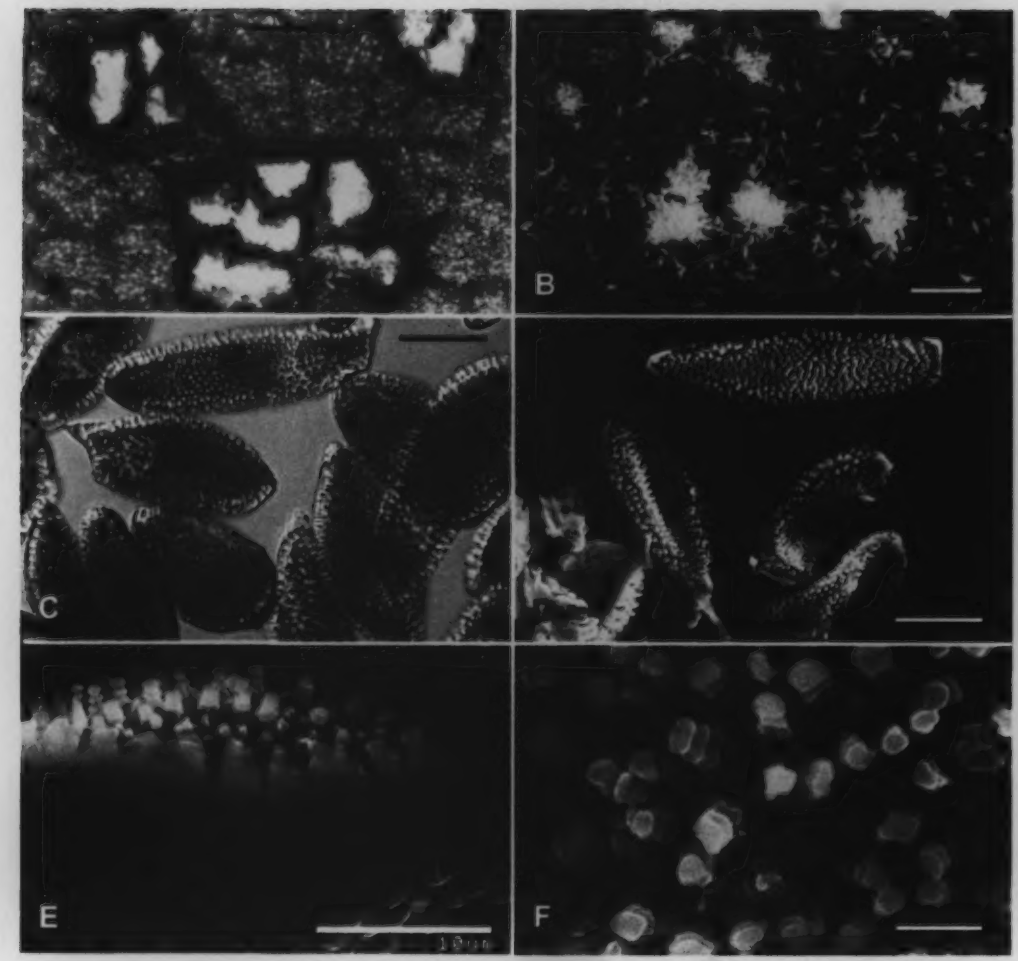
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Fig. 2.16. *Chrysomyxa piperiana* on *Picea sitchensis* (California, PUR 48213). (A) Infected needles showing spermogonia (short arrow) and tubular aecia (long arrow). (B–D) Aeciospores: (B) by LM, (C) single spore by SEM, and (D) details of surface ornamentation. (E, F) Aecial peridium: (E) smooth outer surface, and (F) shallowly warted inner surface. Bar in A = 2.5 mm; in B, C, E, F = 20 μm; in D = 2 μm.

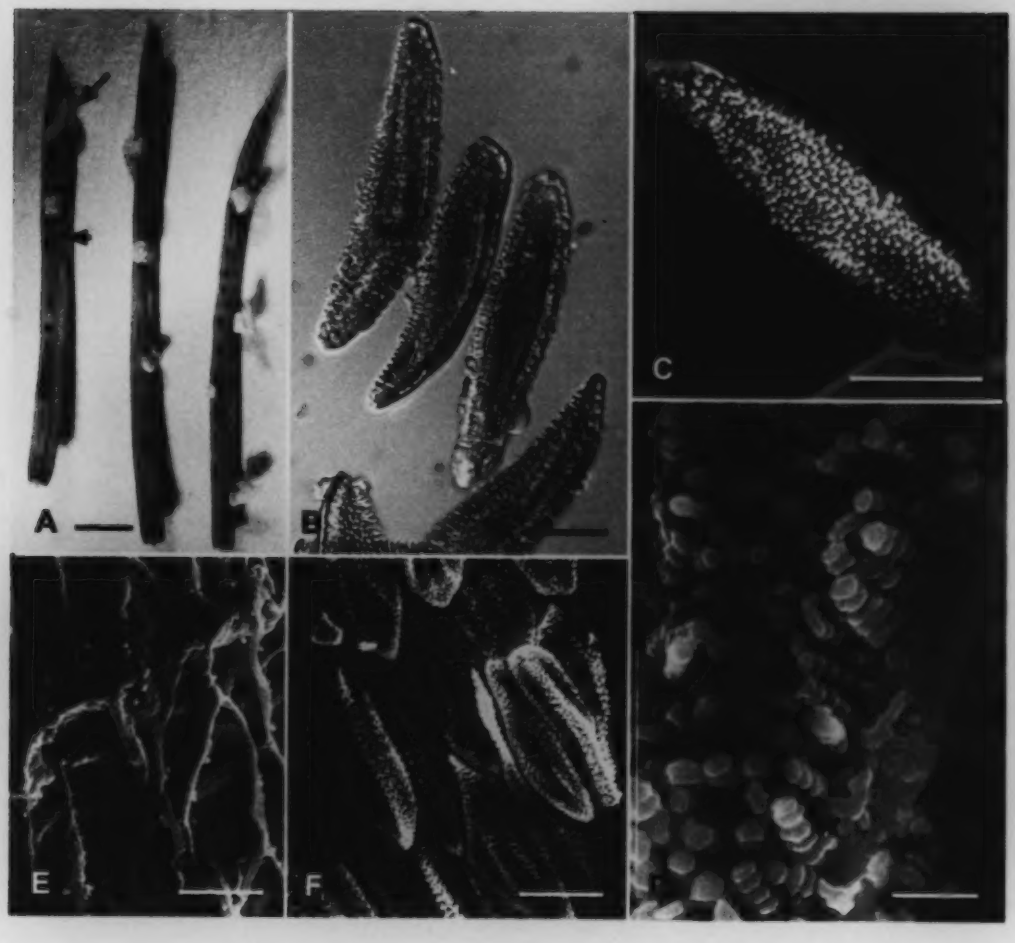
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Fig. 2.17. *Chrysomyxa pirolata* on *Pyrola* spp. (A–E) On *P. asarifolia*, central Alberta. (A) Cross section of telium, showing constricted base composed of chains of narrow colorless cells (arrow) and distal pigmented teliospores. (B) Cross section of cupulate uredinium containing urediniospores. (C–E) Urediniospores with typical pulvinate warts. (F–H) Urediniospores with less common elongated warts, on *P. virens*, northern Alberta. Bars in A, B, D, G =10 μm; in C, F = 20 μm; in E, H = 2 μm.

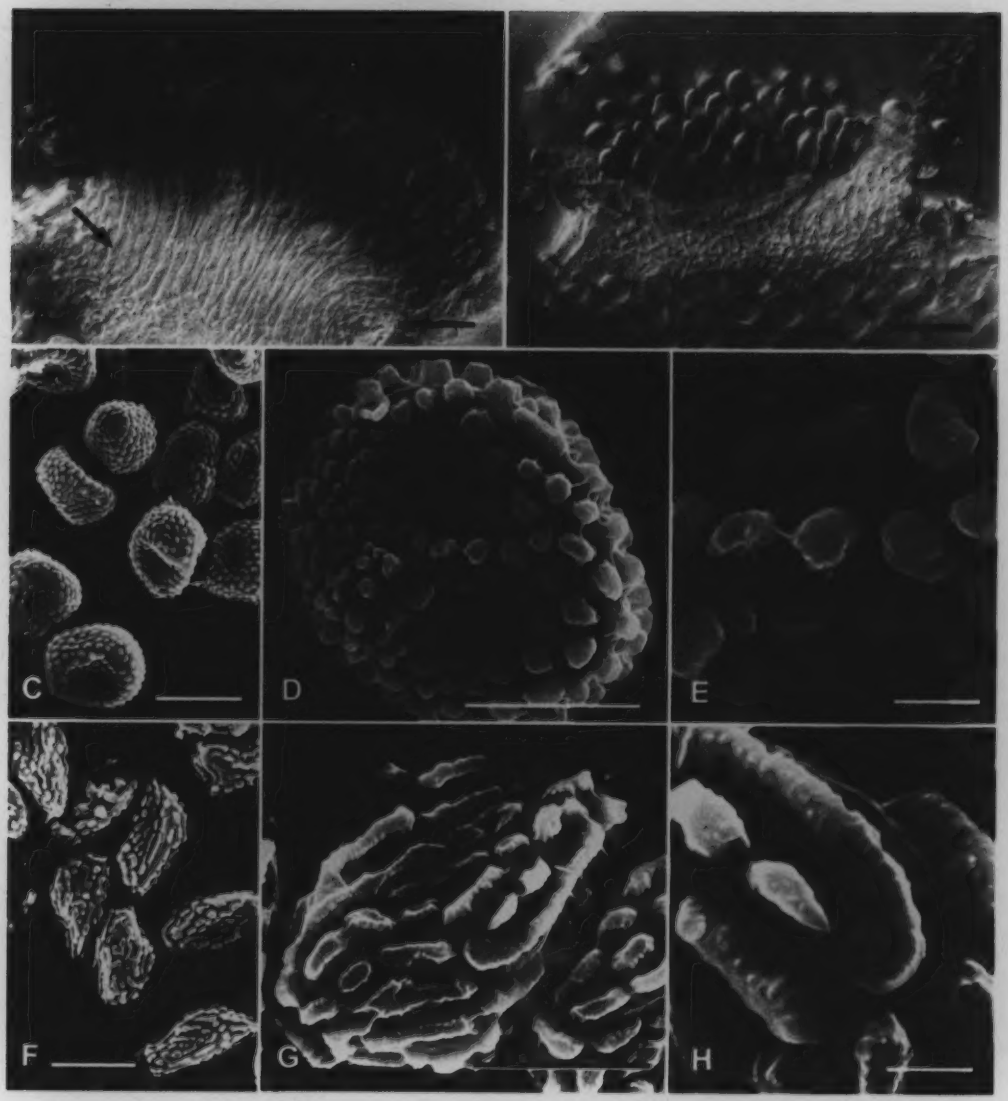
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Fig. 2.18. *Chrysomyxa pirolata* on *Picea* spp. (A) Healthy (arrow) and infected (arrowhead) cones, central Alberta, late July. (B) Cross section of cone scale with subepidermal, indeterminate spermogonium. (C–F) Aeciospores: (C) group of spores with occasional elongated warts, (D) single spore, (E) annulate warts with thin basal connections, (F) by LM. (G) Aecial peridial cells. (H) Peridial cell next to much smaller aeciospore. Bars in A = 2 cm; B = 40 μm; C, F–H = 20 μm; D = 10 μm; E = 2 μm.

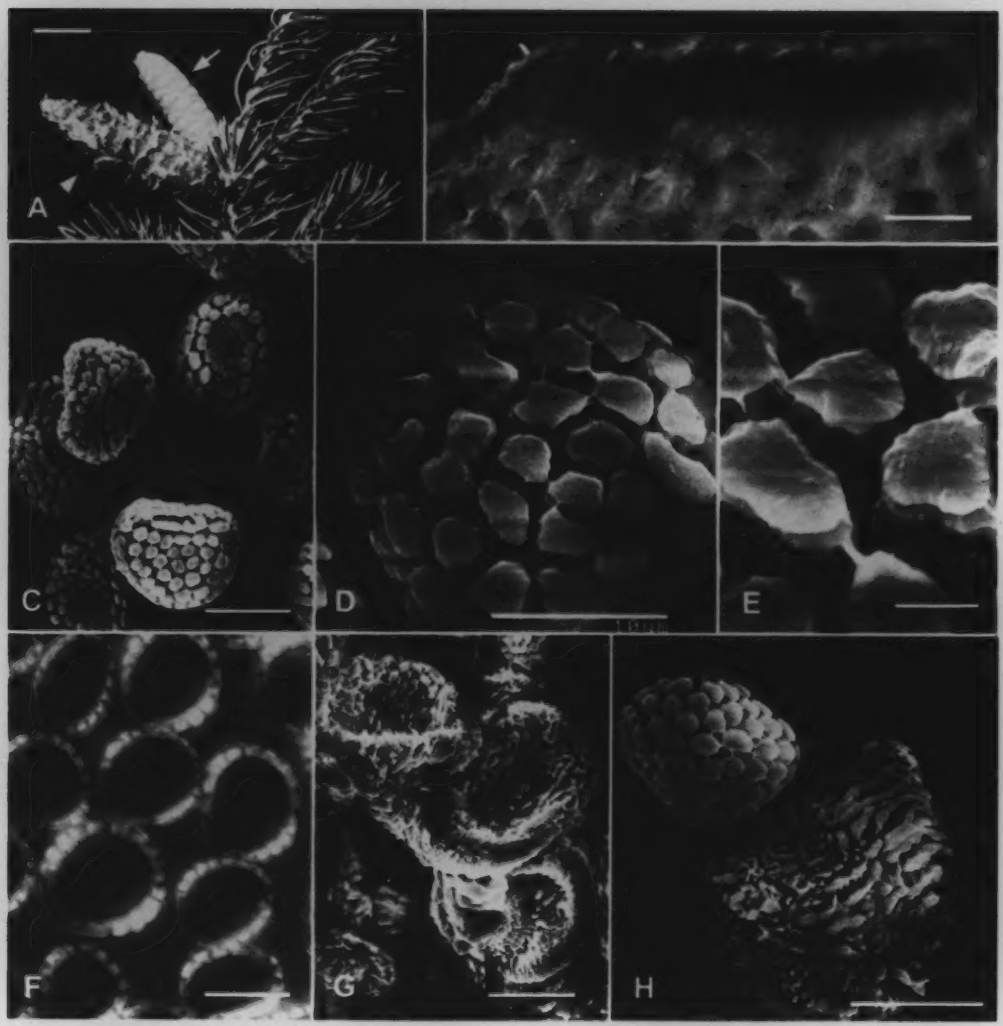
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Fig. 2.19. *Chrysomyxa reticulata*. (A) Urediniospores of *C. reticulata* and *C. nagodhii* (arrow) found on the same *Ledum* leaf (Nova Scotia, PUR 4866). (B–E) Urediniospores: (B) by LM, (C) from *L. groenlandicum*, northern Alberta (CFB 7334), (D) from cultivated *Rhododendron* sp., Washington (PUR 54533), (E) details of surface ornamentation. (F, G) Aeciospores from artificial inoculation of *Picea glauca*: (F) spores with cap having central groove, (G) details of surface ornamentation. (H) Cell of aecial peridium (arrow), among aeciospores, showing concave outer surface. (I) Inner surface of peridial cells. Bars in A, B, H = 20 μm; in C, D, F, I = 10 μm; in E, G = 2 μm.

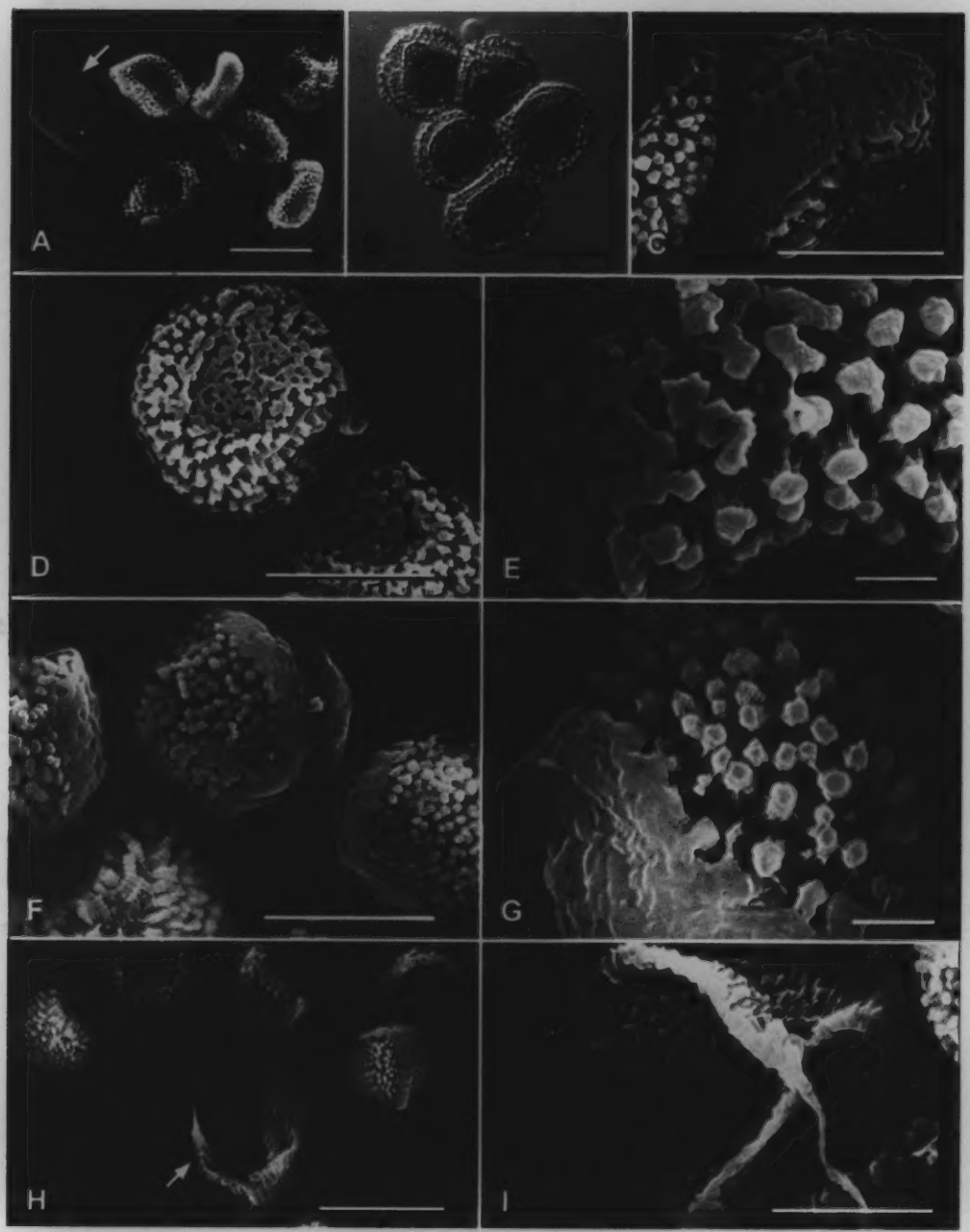
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Fig. 2.20. *Chrysomyxa rhododendri.* (A–C) Urediniospores from *Rhododendron lapponicum*, Manitoba (PUR 53201): (A) by LM, (B) single spore by SEM, and (C) details of surface ornamentation. (D–F) Aeciospores from *Picea abies*, Switzerland (CFB 22181): (D) by LM, showing small caps at ends, (E) by SEM, and (F) details of surface ornamenation. (G, H) Aecial peridium: (G) shallowly concave, smooth outer surface of cells, (H) inner cell surface, showing labyrinthine appearance of warts. Bars in A, D, G, H = 20 μm; in B,E = 10 μm; in C, F = 2 μm.

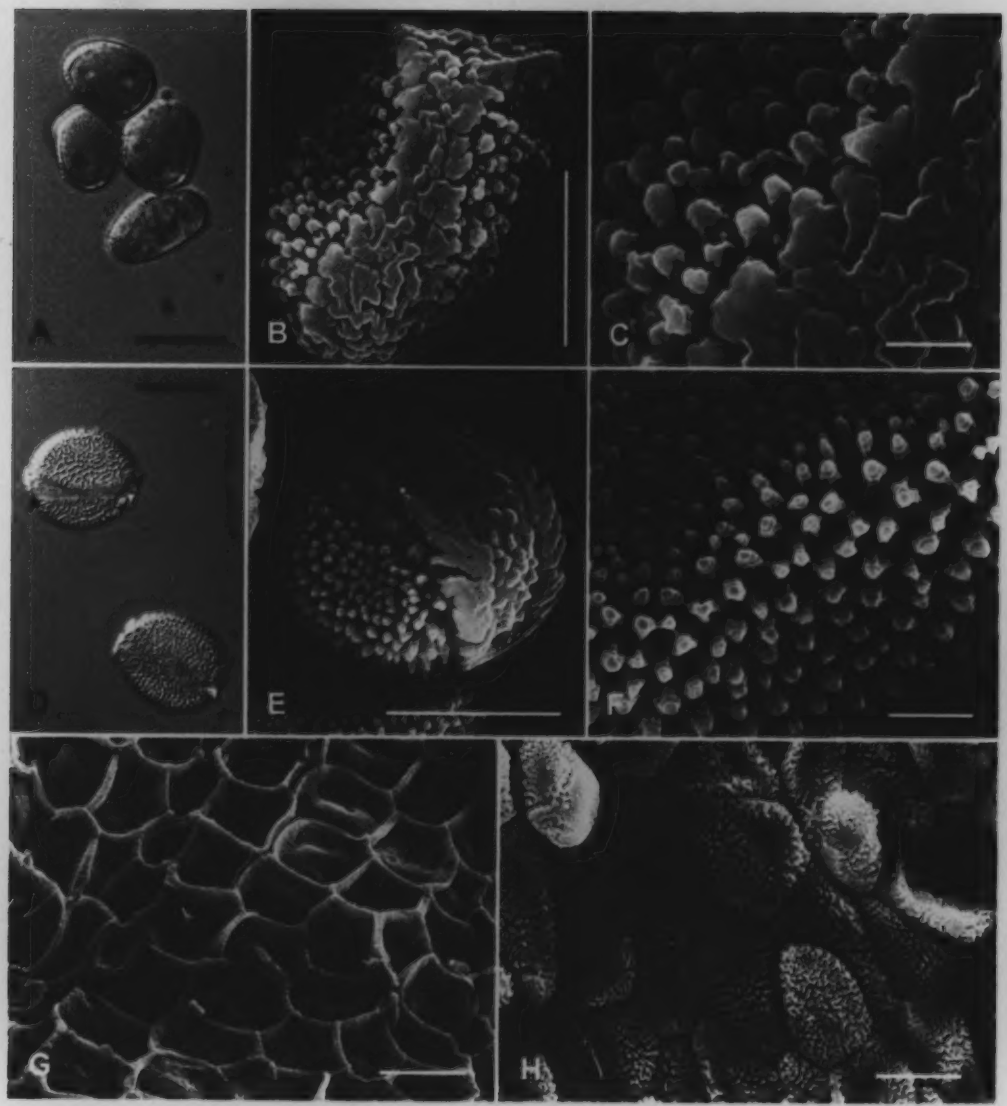
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Fig. 2.21. *Chrysomyxa roanensis* (Tennessee, BPI 140970, 140973). (A) Cross section of telium on underside of leaf of *Rhododendron punctatum*. Teliospores separate easily. (B) Chain of monokaryotic teliospores. (C–E) Urediniospores: (C) by LM, (D) single spore with warted longitudinal cap, and (E) broken edge of cap and broad warts. (F–H) Aeciospores: (F) by LM, (G) single spore with some dehiscent warts (lower part), (H) irregularly shaped warts, sometimes joined laterally. (I, J) Aecial peridium: (I) outer surface, (J) warted inner surface. Bars in A = 50 μm; in B = 12 μm; in C, F, I, J = 20 μm; in D, G = 10 μm; in E = 5 μm; in H = 2 μm.

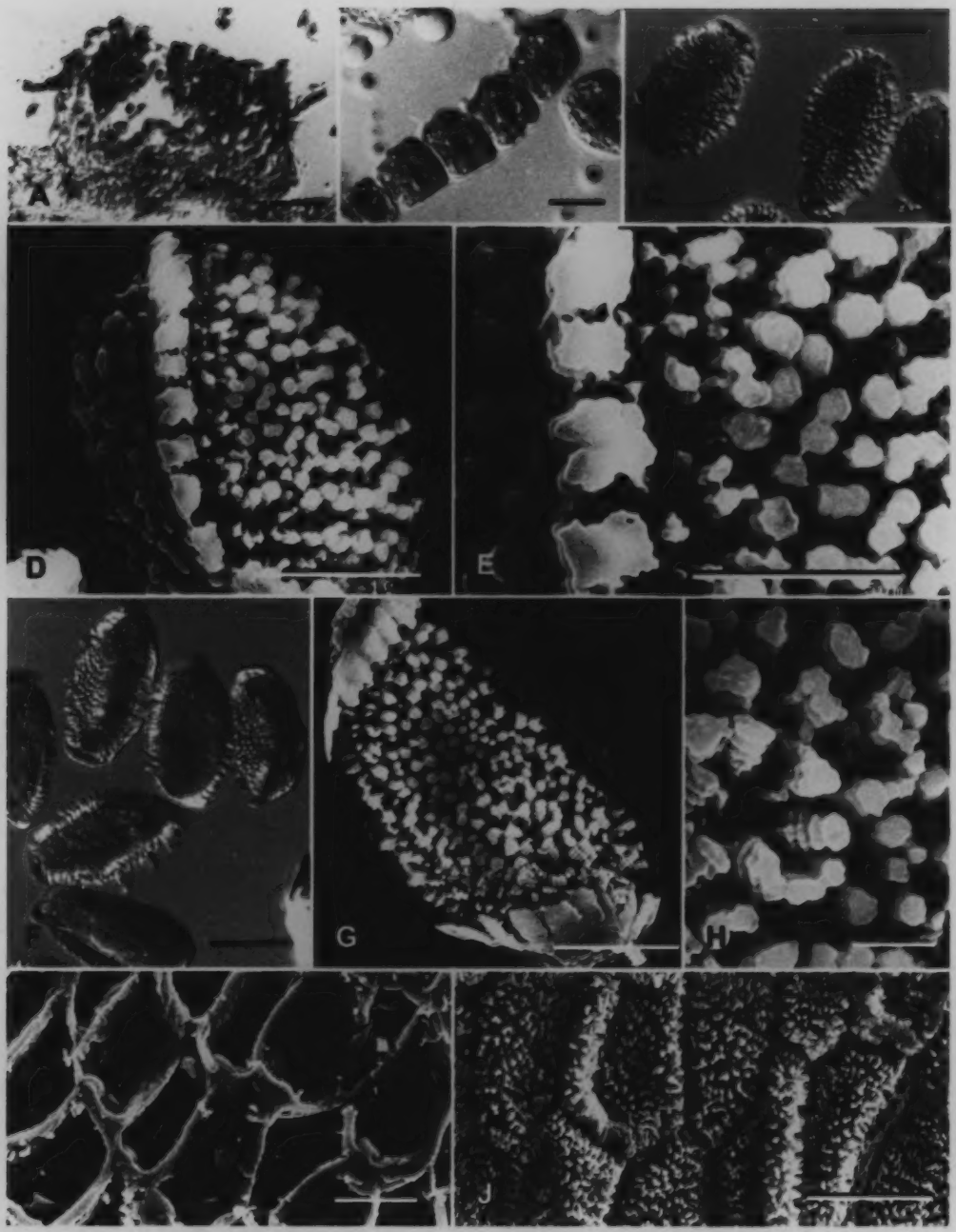
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Fig. 2.22. *Chrysomyxa vaccinii* on *Vaccinium parvifolium* (Queen Charlotte Islands, B.C., PUR 54528). (A–C) Urediniospores: (A) by LM, (B) single spore by SEM, and (C) irregular surface ornamentation. Bar in A = 20 μm, in B = 10 μm, in C = 2 μm.

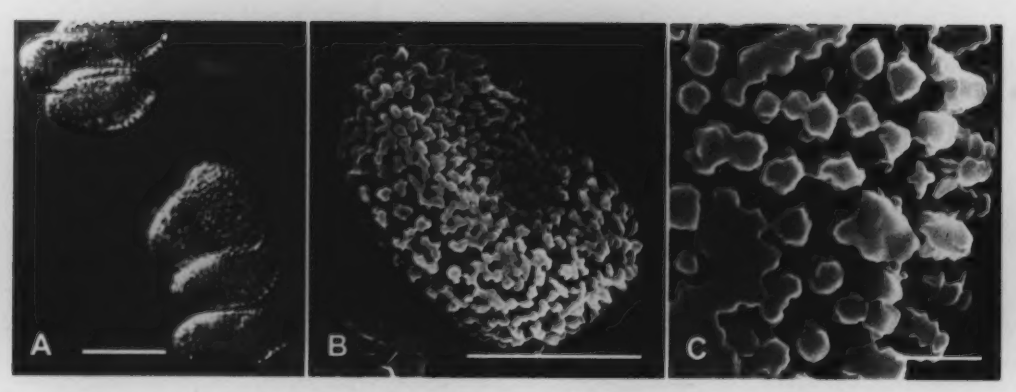
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Fig. 2.23. *Chrysomyxa weirii* on *Picea glauca*. (A) Year-old spruce needle bearing mature telium; note chlorotic banding. Bar = 1 mm. (B) Teliospores dispersed in water. Bar = 20 μm. (C) Germinated teliospore (arrow) with basidium producing two basidiospores (arrowheads). Bar = 6 μm.

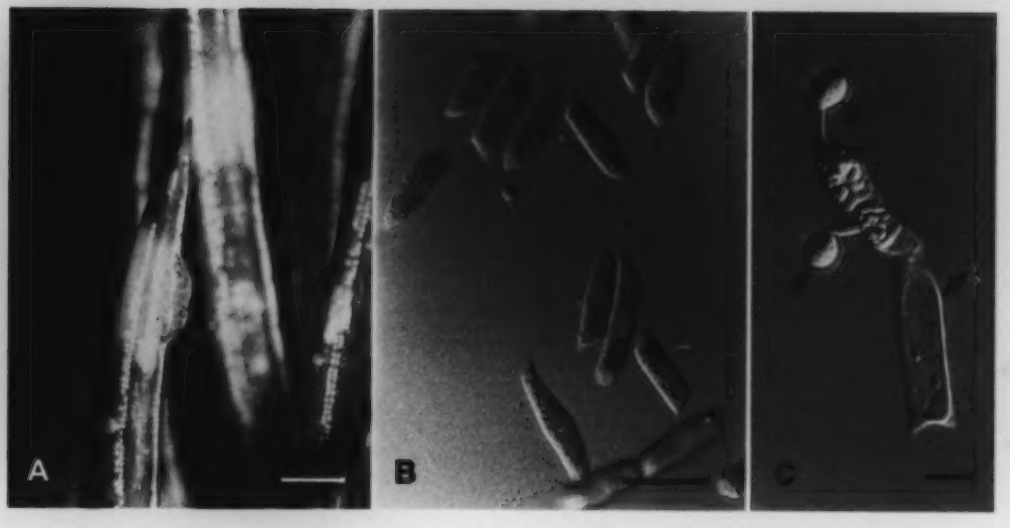
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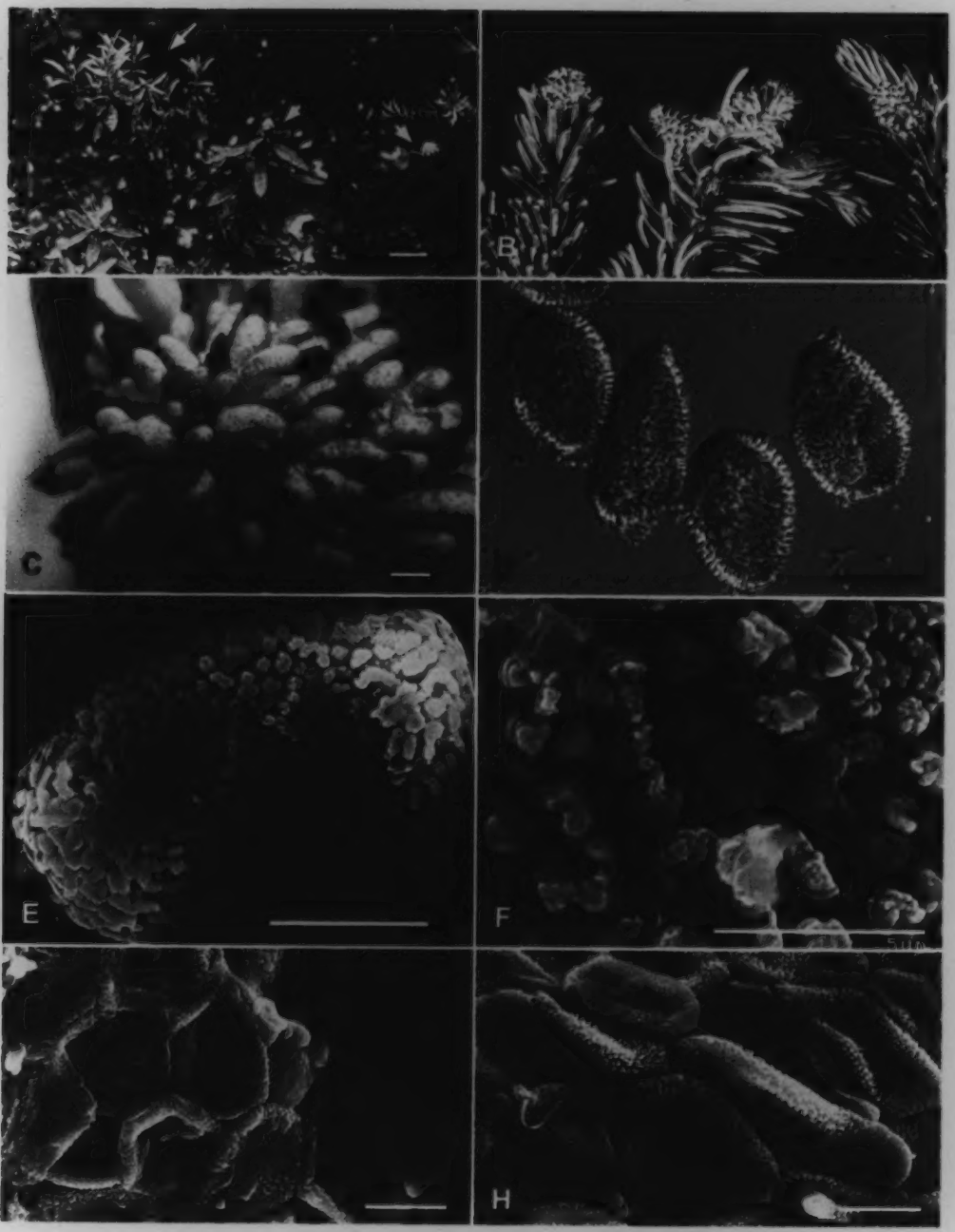
Fig. 2.24. *Chrysomyxa woroninii*. (A) Perennially infected witches' broom (long arrow) and healthy shoots (short arrows) of *Ledum groenlandicum*, north of Hinton, Alberta. Several spruce buds less than 1 m away were infected (not shown). (B) Several systemically infected spruce buds. (C) Closer view of infected spruce bud showing unopened elongated aecia on stunted needles. Arrow indicates tiny dotlike spermogonia at the end of a needle. (D–F) Aeciospores: (D) by LM, (E) single spore by SEM, and (F) irregularly shaped warts, sometimes joined laterally. (G, H) Aecial peridium: (G) outer surface, (H) inner surface. Bar in A = 4 cm; in B = 1 cm; in C = 1 mm; in D, G, H = 20 μm; in E = 10 μm; in F = 5 μm.


B
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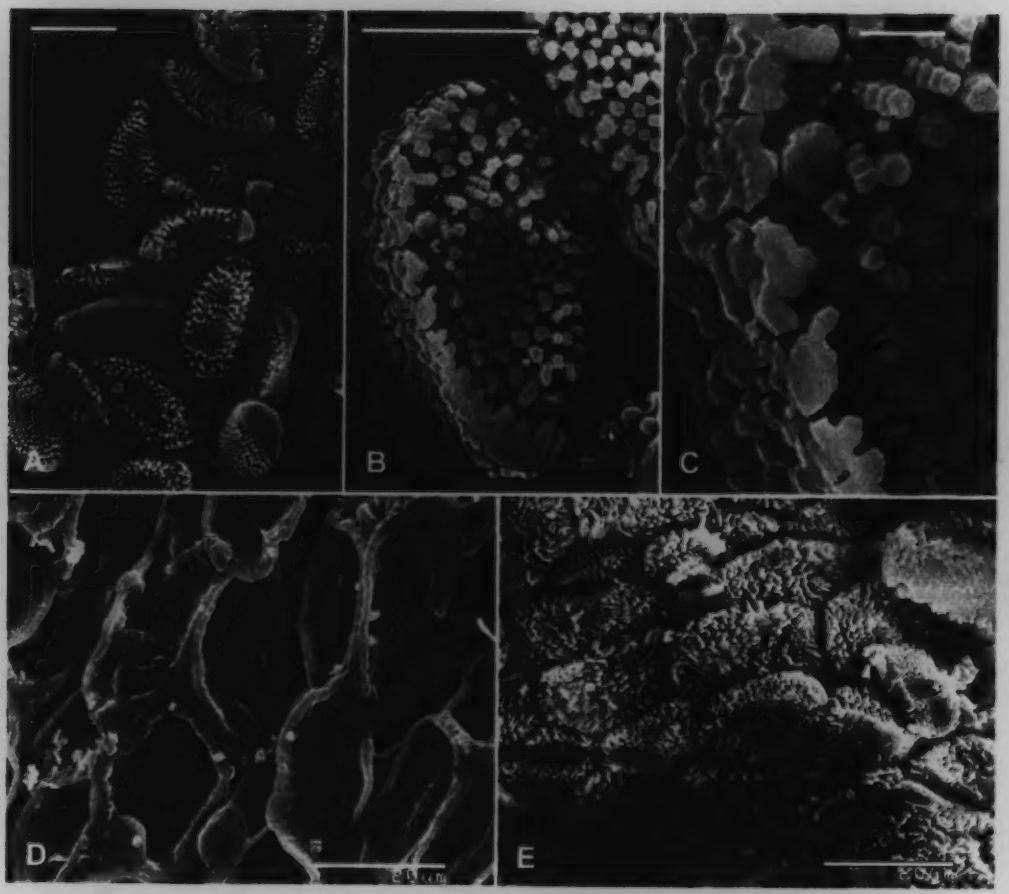
H

Fig. 2.25. *Peridermium zilleri* on *Picea sitchensis*. (A–C) Aeciospores: (A) group of elongated spores; (B) single spore with smoother cap over part of spore; (C) details of cap and annulate warts with broad tops. (D, E) Aecial peridium: (D) outer surface; (E) densely warted inner surface. Bars in A, D, E = 20 μm; in B = 10 μm; in C = 2 μm.


A
B
C
D
E

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Chapter 3. The use of molecular characters in the systematics of *Chrysomyxa*

INTRODUCTION

The rust fungi (Uredinales) present unique challenges for study at the molecular level. Because they are obligate parasites, only a few species have been successfully grown on artificial media, dramatically restricting the amount of DNA that is available and thus limiting the techniques that can be used for molecular phylogenetic analysis. For rust fungi, it is usually necessary to obtain DNA from field-collected infected host plants or artificially inoculated greenhouse-grown plants, but in either case the potential for contamination is great. This problem has been largely overcome by the use of rust-specific primers to amplify the DNA (Gardes and Bruns 1993); however, different rust species may be present on adjacent or even the same plant, and therefore material for DNA extraction must be carefully chosen.

Nuclear ribosomal DNA (rDNA) sequences have been widely used in recent systematic studies of the Uredinales. This region of the genome occurs in multicopy, tandem arrays that evolve concertedly, thus improving the likelihood that sufficient DNA will be amplified for sequencing (Vogler and Bruns 1993; Zambino and Szabo 1995). The two ITS regions are highly variable compared with the more highly conserved sequences of the flanking 28S or 18S regions, and therefore is particularly useful for intragenus comparisons. In studies of rust fungi, ITS sequences have provided new information in several areas: to construct phylogenies among genera (Sjamsuridzal et al. 1999), to determine species relationships within a single genus (Nakamura et al. 1998*a*, *b*; Vogler and Bruns 1998; Zambino and Szabo 1993), to support the connection of teleomorphic and anamorphic states (Vogler and Bruns 1993), and to confirm the close relationship of morphologically similar autoecious and heteroecious ('correlated') species (Zambino and Szabo 1993, 1995).

Species in the genus *Chrysomyxa* (Uredinales, Coleosporiaceae) are a group of closely related obligate parasites confined mostly to *Picea* spp. and alternating to several

angiosperms, mostly genera in the Ericaceae *s.l.* Overall morphology is very similar among species, and host affinities and subtle morphological differences are used to distinguish among them. Little is known about the evolutionary history of these rusts. It is speculated that they coevolved with their hosts, for which the phylogenetic history is much more widely known, based on fossil records (Miller 1977, 1988; Nixon and Crepet 1993; LePage 1993; Tiffney and Wang 2000) and morphological- and molecular-based phylogenetic analyses (Spethmann 1987; Anderberg 1992, 1993, 1994*a*, *b*; Cullings and Bruns 1992; Cullings 1994; Judd and Kron 1993; Kron 1996, 1997; Kron and Chase 1993; Kron and Judd 1990; Kron et al. 1999). GenBank contains only one published sequence of a *Chrysomyxa* species, *C. arctostaphyli*, which was used as an outgroup for a phylogenetic analysis of pine stem rusts (*Cronartium* spp. and *Peridermium* spp.) (Vogler and Bruns 1998). DNA-based techniques could contribute significantly to an understanding of the relationship among *Chrysomyxa* species and of the relationship of the whole genus to other genera of the Uredinales. They might also elucidate the life cycles of species for which information is lacking, for example, whether *Peridermium zilleri* is the aecial state of *C. vaccinii* (Chapter 2). DNA analysis would also provide independent characters to test the significance of minor morphological differences among species, or within species with large geographical variation, such as *C. ledicola* (Chapter 2). Moreover, DNA sequence information would provide characters that could be combined with morphological data to test the hypothesis that individual species have coevolved with their ericaceous hosts.

This study reports the results of preliminary investigations into suitable methods for the extraction and amplification of DNA from several species of *Chrysomyxa* occurring in Europe and/or North America and the use of sequencing of the ITS regions of rDNA for phylogenetic studies of the genus.

MATERIALS AND METHODS

DNA extraction and amplification were attempted for 12 species of *Chrysomyxa* and for *Endocronartium harknessii*, a pine stem rust, for comparison. Because spores of

these fungi are only available at very limited times during the year, it was usually necessary to store spores or specimens for varying lengths of time before DNA extraction. The species and samples are described, along with the storage method and age before use, in Table 3.1.

DNA extraction

Two methods of obtaining DNA from aeciospores or urediniospores were evaluated, as follows.

(1) DNA was extracted according to the chloroform/isopropanol protocol of Gardes and Bruns (1993), with minor modifications. CTAB extraction buffer (20 μL) was added to <5 mg of spores in a 1.5-mL centrifuge tube. The mixture was alternately frozen in liquid nitrogen and thawed in a 65°C water bath. When frozen for the fourth time, the material was ground with a clean micropestle, vortexed, frozen, and ground again. Additional extraction buffer (400 μL) was added, the material was frozen and ground again, then vortexed. Tubes were incubated in a water bath at 65°C, then vortexed again. Chloroform (600 μL) was added to remove proteins, and the tubes centrifuged at room temperature for 15 min at 13 000 rpm. The upper phase was transferred to a new tube, then 600 μL of isopropanol was added to precipitate DNA. Tubes were placed in a freezer for 10 min, centrifuged for 10–15 min, and the supernatant discarded. The DNA pellet was washed twice with 600 μL of cold 70% ethanol, and dried in a vacuum centrifuge. DNA was resuspended in sterile water and cleaned using Millipore Ultrafree-MC filter units. Samples were stored at –20°C before amplification.

(2) DNA was extracted from spores crushed between glass slides (Taylor and Swann 1994, with modifications). To render slides water-repellant, they were dipped in 5% dimethyldichlorosilane in chloroform and air-dried. Before use, slides were cleaned in concentrated HCl, rinsed in sterile water and air-dried. A small quantity of spores (from one aecium or several uredinia on the same leaf) were placed in the center of a slide. A second slide was placed on top of the first, and pressure was applied to crush the spores. The slides were separated and 20 μL of TE buffer (10 mM Tris-HCl / 0.1 mM

Table 3.1. Specimens used for DNA extraction and amplification

Species	No.[a]	Host	Spore stage	Storage method
C. arctostaphyli	22048	*Picea glauca*, AB	Aeciospores	Dried 2 1/2 yr
	22190	*P. glauca*, AB	Aeciospores	Frozen 7 mo
C. cassandrae	22069	*Chamaedaphne calyculata*, AB	Urediniospores	Frozen 1 1/2 yr
	DAVFP 14652	*C. calyculata*, BC	Urediniospores	Dried 38 yr[b]
C. empetri	---	*Empetrum nigrum*, AB	Urediniospores	Frozen 2 yr
C. ledi	22061	*P. abies*, Finland	Aeciospores	Dried 3 1/2 yr
C. ledicola	---	*L. groenlandicum*, AB	Urediniospores	Frozen 5 mo
	---	*P. glauca*, AB	Aeciospores	Frozen 7 mo
C. nagodhii	22137	*Ledum groenlandicum*, AB	Urediniospores	Dried 1 3/4 yr
C. neoglandulosi	22174	*L. glandulosum*, AB	Urediniospores	Dried 1 1/2 yr
C. pirolata	22049	*P. glauca*, AB	Aeciospores	Frozen 1 1/2 yr
	22050	*P. glauca*, AB	Aeciospores	Frozen 3 1/2 yr
	22145	*P. glauca*, AB	Aeciospores	Dried 1 3/4 yr
C. reticulata	22183	*L. groenlandicum*, AB	Urediniospores	Dried 9 mo
	DAVFP 10764	*Rhododendron* sp., BC	Urediniospores	Dried 41 yr[b]
C. rhododendri	22181	*P. abies*, Switzerland	Aeciospores	Frozen 6 mo
C. vaccinii	22093	*Vaccinium parvifolium*, BC	Urediniospores	Dried 42 yr[b]
C. woroninii	22014	*P. glauca*, AB	Aeciospores	Frozen 7 mo
	22172	*P. mariana*, AB	Aeciospores	Dried 9 mo
E. harknessii	---	*Pinus contorta*, AB	Aeciospores	Frozen 1 ½ yr

[a]CFB, unless otherwise indicated.
[b]DNA could not be extracted.

EDTA, pH 8.0) was added to the crushed spores and the pipette was used to mix the spores and buffer. The liquid was carefully sucked up with the pipette and placed in a 1.5 μL centrifuge tube; the process was repeated with another 20 μL of buffer and this was added to the first. Because of the volatile nature of rust spores, extractions were carried out in a separate room from amplification. To prevent cross-contamination of samples, Aeroseal pipette tips were used, and between extractions of different samples, gloves were changed and table surfaces and pipetters were wiped with disinfectant. Samples were incubated in a 65°C water bath for about 30 min and used immediately for amplification.

Amplification

Because the amount of total DNA obtained from each species was highly variable, each sample of total DNA was diluted from 1:10 to 1:5000, and several different dilutions were used as template for each species. A portion of rDNA including both ITS spacer regions and the 5.8S subunit was amplified by the polymerase chain reaction (PCR), using the primers ITS1-F or ITS1, and ITS4 (Gardes and Bruns 1993; White et al. 1990) (Fig. 3.1). Reaction volumes of 100 μL contained 10 μL PCR buffer, 2.5 mM $MgCl_2$, 50 μM each of dGTP, dATP, dTTP, and dCTP (Boehringer Mannheim Biochemica), 0.4 μM of each primer, 1 unit of *Taq* DNA polymerase, and 40 μL of DNA template. A negative control containing all ingredients except template DNA was used for each set of reactions. PCR amplifications were carried out on a DNA Thermal Cycler (Gene E. Techne Ltd., Princeton, New York) using the following parameters: 93°C denaturation for 1 min, 52°C annealing for 1 min, 72°C extension for 2 min. The total number of cycles was 35 with an initial denaturation step of 2 min at 93°C and a final extension at 72°C for 7 min. Amplified PCR products from different dilutions of DNA template were combined and concentrated and cleaned using Wizard PCR Preps columns (Promega, Madison, Wisconsin), following the manufacturer's instructions. Products were verified by gel electrophoresis and viewed under ultraviolet light after staining with ethidium bromide.

Sequencing

Automated DNA sequencing reactions were performed using standardized methods, then processed and analysed on an ABI 373A automatic DNA sequencer (Perkin-Elmer: Applied Biosystems, Foster, California) following the protocols suggested by the manufacturer. Sequences of complementary strands were determined using the primers ITS-1 or ITS-lF, and ITS-4. Rust-specific primers ITS2-R and ITS3-R, which anneal in the 5.8S region, were also used to verify the sequence of one species (Fig. 3.1).

RESULTS AND DISCUSSION

DNA was obtained from both dried herbarium specimens and frozen material; however, more DNA was usually obtained from frozen spores. A much larger number of spores was required to obtain detectable DNA from dried specimens older than 1 year, but no detectable DNA could be extracted from very old herbarium specimens (38–42 yr) (Table 3.1). The slide-crushing method of extracting DNA from small quantities of rust spores was far superior to extraction with the chloroform/isopropanol protocol. With the latter method, the tiny pellet of DNA obtained was very difficult to work with and was frequently lost during the cleaning process. Using the slide crushing method and fresh or frozen material, adequate DNA could be extracted from a small number of aeciospores or urediniospores (about 100–200). However, because the extracted DNA in buffer was not cleaned to remove enzymes and other cell components, DNA degraded very quickly, and it was necessary to carry out PCR reactions immediately. Even when samples were stored at –20°C, amplifications were unsuccessful 1 wk later.

The ITS region was found to be of limited usefulness for systematic studies of the genus *Chrysomyxa*. Six species in the genus (*C. pirolata*, *C. arctostaphyli*, *C. ledicola*, *C. woroninii*, *C. nagodhii*, and *C. reticulata*) plus *Endocronartium harknessii* were sequenced, but readable sequences were obtained for only *C. empetri* and *E. harknessii*. For some species, sequences were completely ambiguous (double peaks obtained); for other species, parts of sequences were unambiguous, but others regions could not be read. For a given species, sequencing of the complementary strand showed deterioration at

exactly the same locations in the ITS region. This suggests that the ambiguity did not result from cross-contamination of samples, but that the ITS region is variable within a single sample. The variation is likely the result of very small changes in the sequence among nrDNA copies, because double bands could not be detected by gel electrophoresis of the amplified DNA.

The length of the amplified region of *C. empetri* was 612 bp. When compared with the ITS region of *C. arctostaphyli* (GenBank accession No. L76488), the similarity was 91% (Fig. 3.2).

Variation of the ITS region of a single individual has been observed in Pinaceae and several other plants (Buckler et al. 1997; Gernandt and Liston 1999; Gernandt et al. 2000) and in some fungi. In *Larix* and *Pseudotsuga* this polymorphism has been attributed to hybridization, polyploidy, or a slow rate of concerted evolution among nuclear rDNA loci. In fungi such as *Fusarium sambucinum* Fuckel, distinct ITS types have been observed in different strains of this species that show greater variation than one would expect from different species (O'Donnell 1992). It has been suggested that these ITS types have recently diverged, or that because this is largely an asexual species, there has been little opportunity for homogenization of the ITS region during meiotic recombination. The source of variation in the ITS region of *Chrysomyxa* species is unknown. In some cases, hybridization of different species may occur, but little morphological evidence of hybrids of co-occurring species was found in the study of European and North American species in the genus (Chapter 2). Savile (1969) claimed to have found hybrids of the rusts that infect *Ledum* spp. in western Canada, but, when examined by SEM, several of these purported hybrids were consistent with a single species.

ITS variation confounds the ability to infer species phylogeny from a gene phylogeny. The use of another region of the rDNA for sequence analysis of *Chrysomyxa* needs to be investigated. Although the 28S (large subunit) and 18S (small subunit) of rDNA may be too conserved for species-level comparisons, the 5' end of the 28S has been useful at this level for some other fungi (e.g. Mori et al. 2000).

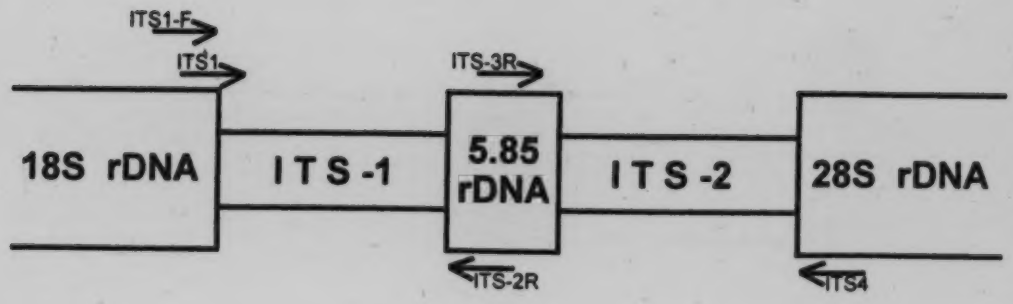

Fig. 3.1. Diagram of a portion of the rDNA unit repeat showing coding and spacer (ITS) regions, and the annealing sites of the primers used for PCR and sequencing.

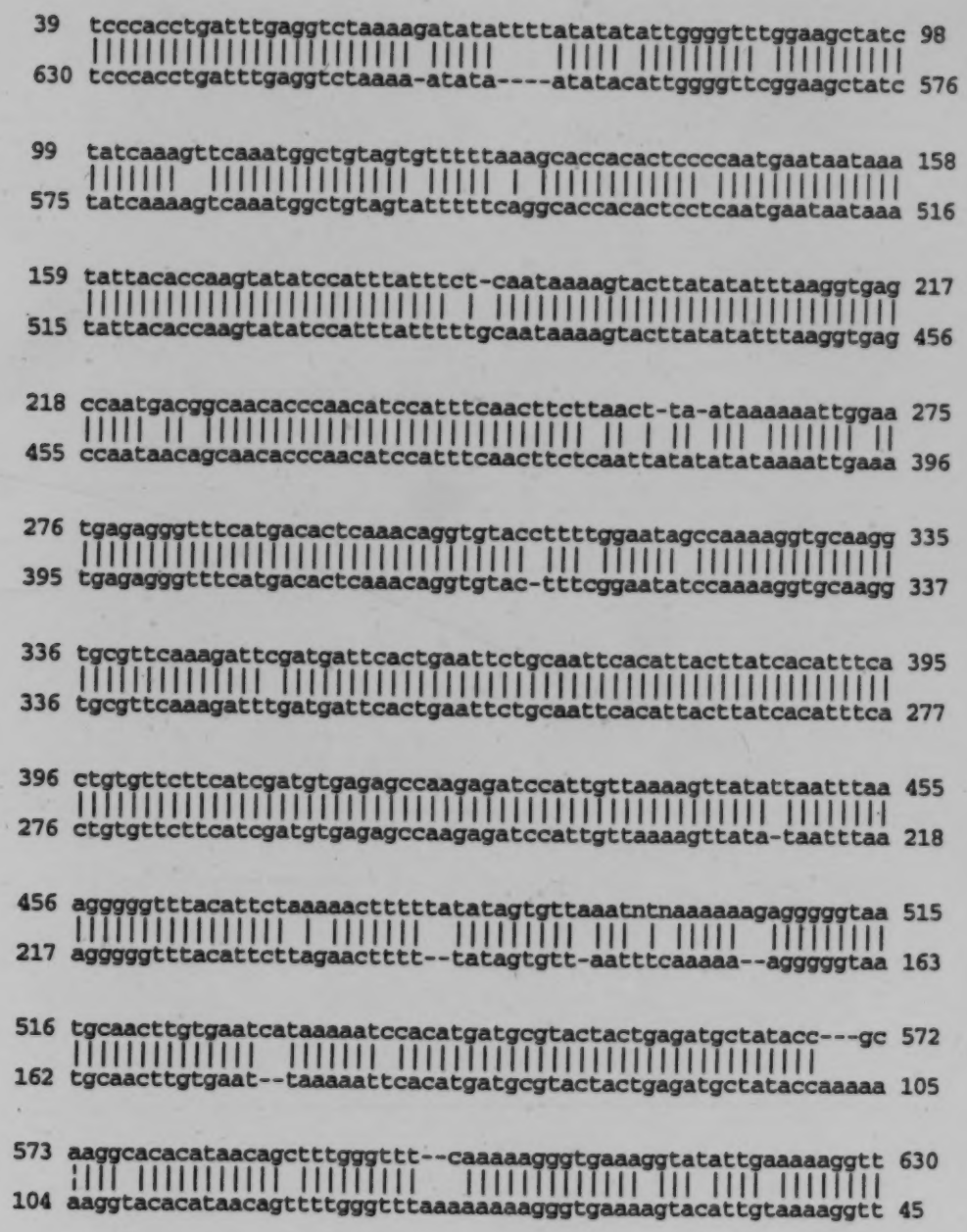

Fig. 3.2. Comparison of the sequence of the ITS region of *C. empetri* (top) with a published sequence of *C. arctostaphyli*. The species are identical at 91% of sites.

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PART II. BIOLOGICAL STUDIES OF SELECTED SPECIES OF CHRYSOMYXA

Chapter 4. Clarification of the life cycle of *Chrysomyxa woroninii* on *Ledum* and *Picea*[1]

INTRODUCTION

Chrysomyxa woroninii Tranz. (Uredinales) is the name given by Tranzschel (1903) to a fungus that systemically infects *Ledum palustre* L. in Europe. The rust is confined to far northern or subalpine regions of Canada and Alaska, Europe, Siberia, Kamchatka, Japan, and China (Savile 1950; Kuprevich and Tranzschel 1957; Gäumann 1959; Spaulding 1961; Wood 1986; He et al.1995). On *Ledum* spp., *C. woroninii* causes witches' brooms, and in early spring the new leaves on the brooms bear hypophyllous telia. Bud rust of spruce (*Picea*), caused by *Peridermium coruscans* Fr., is the presumed aecial state of *C. woroninii*. In early spring the newly opened spruce buds, which are systemically infected, produce spermogonia and later aecia on the stunted needles. This disease may retard growth of heavily infected seedlings in spruce regeneration areas. In Alaska, it has also been found on female spruce cones. Because infected cones do not produce viable seeds, the disease may reduce spruce regeneration near treeline (McBeath 1981, 1984).

Chrysomyxa woroninii is assumed to be heteroecious because of the close proximity in the field of systemic bud infections on spruce and witches' brooms on *L. palustre* in Europe (Kuprevich and Tranzschel 1957) and on *L. palustre* var. *decumbens* Ait. and *L. groenlandicum* Oeder in northern Canada (Savile 1950, 1955). The consistent proximity of spruce bud rust to infected *Ledum* spp. was also observed in the Northwest Territories (Y. Hiratsuka, personal communication), northern Finland, and western Alberta by the author. In 1996, infections were common on both hosts in a subalpine zone 67 km north of Hinton, Alberta, near the Little Berland River (53°40' N 118°15'W).

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[1]A version of this chapter has been published: Crane, P.E., Hiratsuka, Y., and Currah, R.S. 2000. Mycol. Res. 104: 581–586.

These collections are the first record of this rust on *L. groenlandicum* in central Alberta, although bud rust was occasionally collected on spruce in central and northern Alberta (Robins et al.1964, 1972, 1974). Elsewhere in western Canada it has been found on *Ledum* spp. in northern British Columbia (Wood 1986) and in the Yukon and Northwest Territories (collection records, Mycological Herbarium (CFB), Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada).

In spite of several detailed morphological studies of the spruce bud rust fungus (Kuprevich and Tranzschel 1957; McBeath 1984 ; He et al. 1995), several aspects of its biology are unconfirmed, including its connection with the telia on *Ledum* spp. Previous inoculation attempts to demonstrate the life cycle were unsuccessful (Klebahn 1914) or the results were inconclusive (Liro 1907) because aecia of both *C. ledi* de Bary and *Peridermium coruscans* were produced on spruce. Liro (1907), whose experiments were conducted in the natural habitat of both rusts, concluded that *C. woroninii* is an overwintering form of *C. ledi*.

McBeath (1984) suggested that the bud rust fungus is autoecious and that aeciospores from infected spruce buds cause secondary infections on adjacent shoots later in the season. As further evidence of an autoecious life cycle, she asserted that aeciospore germ tube cytology of the bud rust fungus is similar to that of some autoecious pine stem rusts. He et al. (1995) claimed to have obtained infection of spruce 2 years after inoculation with *C. woroninii* aeciospores from spruce, but no details of their experiments were given.

Other aspects of the life cycle of *C. woroninii* that are in question are the timing and mode of infection in spruce (McBeath 1984; Ziller 1974) and the occurrence of uredinia. Some researchers claim that uredinia do occur (Gäumann 1959; Mäkinen 1964; Gjaerum 1974), whereas others believe that the observed uredinia belong to *C. ledi*, which occurs on the same hosts, but is not systemic (Savile 1950, 1955).

The purpose of this study was to clarify aspects of the life cycle of *C. woroninii*, including the connection between systemic spruce bud infections and systemic shoot

infections in *Ledum* spp., the timing and mode of infection in spruce, and the means of overwintering of the rust fungus.

MATERIALS AND METHODS

Observations of disease cycle

At the Little Berland River site, near Hinton, Alberta, infected black spruce (*Picea mariana* (Mill.) B.S.P.) and *Ledum groenlandicum* were tagged and observed on 25 June, 5 July, 8 Aug., and 4 Oct. 1996, and again on 19 June 1997. Disease symptoms and the presence, time of appearance, and development of all spore states of *C. woroninii* were recorded. Infected plant samples were also collected for morphological and microscopic examination of all stages of the fungus. Dormant buds of *L. groenlandicum* (plus 1 cm of stem) were collected from witches' brooms in October, sectioned, and examined with a light microscope to determine the presence of hyphae. Fresh material was sectioned by hand and mounted in lactophenol/cotton blue or preserved in formalin/acetic acid/ethanol for later processing. The latter were dehydrated through a *t*-butanol series under vacuum, embedded in Paraplast X-TRA (Monoject Scientific) under vacuum at 57 °C, and sectioned (12 µm thick) with a rotary microtome (Jensen 1962). Sections were mounted on microscope slides with Haupt's adhesive (Gurr 1965). Before staining, slides were dewaxed in two changes of xylene. To stain host cells, slides were briefly dipped in aqueous safranin (1%). To stain hyphae, a mixture of aqueous aniline blue in saturated aqueous picric acid (1:5, v/v) was placed on sections with a dropper, warmed gently over a flame until simmering, and washed with distilled water (Cartwright 1929). Slides were dehydrated in an ethanol series to absolute ethanol, then dipped in xylene before mounting in Permount (Fisher Scientific Co.).

A 4-year-old spruce tree and two small *L. groenlandicum* plants infected with the rust were collected in early summer, potted, and kept in a greenhouse in Edmonton, for observation of disease development during the summer 1997. In the fall of 1997, one *L. groenlandicum* plant was moved outdoors to overwinter; snow cover was not ensured. During the second winter (1998 – 1999), the plant was kept covered with snow.

Artificial inoculation

During June and July, 1996 and 1997, several inoculation experiments (Table 4.1) were conducted in an attempt to elucidate the life cycle of *C. woroninii*. Inoculated trees were either grown from seed in a greenhouse or collected from a field location with no known occurrence of *C. woroninii* and inoculated after buds had opened and they were determined to be disease-free. Inoculum consisted of germinating telia on young systemically infected leaves found on brooms of *L. groenlandicum* or of pooled aeciospores obtained from several infected spruce buds collected at the Little Berland River site. Leaves with telia were kept in a moist chamber in a refrigerator at 4 °C to induce basidiospore production. Teliospore germination was confirmed by suspending leaves over glass slides in a moist chamber and observing deposited basidiospores with a light microscope. Two black spruce and 16 white spruce (*P. glauca* (Moench) Voss) were inoculated by laying the *Ledum* shoots, telia side down, onto immature needles of newly opened buds. Trees were misted with distilled water and covered with plastic bags for 48 to 72 h to maintain high humidity. The white spruce were inoculated and kept in a greenhouse at Edmonton, at least 250 km from any known natural occurrence of *C. woroninii*; the two black spruce were inoculated and kept outdoors at the same location. All trees were observed for infection until after needle flush the next spring. Before inoculation of plants with aeciospores, spore viability was tested on 0.3% water agar on glass slides; 1% germination was obtained. Nevertheless, aeciospores were placed onto young shoots of five white spruce and three *L. groenlandicum* with a small paintbrush, then plants were misted, covered as described above, and kept in a greenhouse. In all inoculations, several adjacent non-inoculated plants of the same species and age served as controls.

RESULTS

Yearly disease cycle

A naturally infected spruce kept in the greenhouse had three infected terminal buds producing aecia when it was brought from the field on 5 July. Two subtending buds

Table 4.1. Details of inoculation experiments with *Chrysomyxa woroninii*

Inoculation date	Source of inoculum[a]	Host, (no. of trees), age	Symptoms[b]
25 vi 1996	IV, *L. groenlandicum* (CFB 22187)	*P. glauca* (1), 4 yr	–
9 vii 1996	IV, *L. groenlandicum* (CFB 22188)	*P. glauca* (2), 3 yr	–
		P. glauca (13), 7 weeks	Some needle discoloration
	I, *P. mariana*	*P. glauca* (5), 7 weeks	–
		L. groenlandicum (3), age unknown	–
24 vi 1997	IV, *L. groenlandicum* (CFB 22138)	*P. mariana* (2), 3 or 4 yr	–, + (CFB 22172)

Note: Most experiments were done in a greenhouse at Edmonton, Alberta; the last (with *P. mariana*) was done outdoors.

[a]IV = basidiospores, I = aeciospores. CFB numbers refer to the mycological herbarium of the Northern Forestry Centre, Canadian Forest Service, Edmonton, Alberta, Canada.

[b] –, no infection; +, infection.

appeared uninfected, although one was slightly discolored and the needles were somewhat shrivelled. By 19 July, young aecia had begun forming at the base of these needles, but spermogonia were not observed. One of the field-infected *L. groenlandicum* plants kept in the greenhouse died. The second plant survived overwintering outdoors in spite of the lack of snow cover. The next spring, newly opened leaves on the broom were reddish at the edges, but they did not form mature telia. After the second winter, in which snow cover was provided, the broom produced new leaves covered with hypophyllous telia.

Observations of *C. woroninii* on *L. groenlandicum* and *P. mariana* at the Little Berland River site on five different dates were as follows.

25 June 1996—Small red infected buds were visible on spruce; spermogonia were present only at the ends of needles. Most buds on spruce had not opened. Stunted witches' brooms were found on nearby *L. groenlandicum* (Fig. 4.1). Newly opened leaves on brooms were covered with immature telia on the undersides.

5 July 1996—Many infected spruce buds had turned orange; most aecia were open and shedding spores. On *Ledum* brooms, orange telia (Fig. 4.2) were more obvious on the underside of new leaves than on June 25. Telia and uredinia of both *C. nagodhii* and *C. ledicola* Lagerh. were present on previous year's leaves of both broomed and healthy shoots.

8 Aug. 1996—Infected buds on spruce were drying up and turning black; adjacent buds had discolored and twisted needles. Apart from the brooms there was no sign of *C. woroninii* on *Ledum*. All leaves previously infected with telia had fallen off.

4 Oct. 1996—On spruce, infected buds were black and needles had fallen off below deformed buds for 2–3 cm. *Ledum* plants had 'droopy' leaves, indicating dormancy (Harmaja 1991). Dissection of shoots from brooms showed rust hyphae in the bracts and outer leaves of the bud, but none in the shoot apex or youngest leaves; hyphae were also seen below the bud in the pith and cortex of the stem (Fig. 4.3). Hyphae were intercellular, contained deep yellow vacuoles, and were 6–9 µm wide. Hyphae in the

dense tissue near the bud and in the young undeveloped leaves were convoluted and irregular in shape (Fig. 4.4). Haustoria were simple (unbranched) and vesicular.

19 June 1997—Tagged *Ledum* plants were again producing new leaves with young telia on brooms. Spruce buds that were infected in 1996 were blackened and had no needles for some distance below the old bud. There was no new growth on these shoots, and the rust fungus did not sporulate on the same shoots.

Inoculation results

Except for needle discoloration on several seedlings during the first summer, no disease was produced on white spruce or *L. groenlandicum* inoculated with either aeciospores from rusted buds or basidiospores from *C. woroninii* on *Ledum*. Neither did the control plants for any of the experiments show signs of disease. However, two systemically infected buds were produced on one black spruce tree in 1998 from inoculation with basidiospores from *L. groenlandicum* in 1997 (Table 1; Fig. 5.5*a*). Unlike other inoculation attempts, which were done in a greenhouse, this successful experiment was conducted out of doors and the tree was overwintered outside. The first disease symptoms appeared 3 weeks after inoculation (July 1997) as yellow to orange bands on several current-year needles that were exposed to telia on *Ledum* leaves. By late summer, infected needles had fallen from the tree. On 24 Apr. 1998, two yellow, systemically infected buds were visible on the tree. Infected buds opened before the healthy buds on the same tree. By 25 Apr., spermogonia were producing fragrant nectar at the ends of the needles. By 30 Apr., spermogonia were drying up, and needles were becoming swollen and reddish; soon after, aecia began to form along the length of the needles of both buds (Fig. 5.5*b*). One bud remained much smaller than the other, and the center needles died. Typical aecia of *C. woroninii*, however, formed on the rest of the needles of the bud. Morphological characteristics of the spermogonia, aecia, and aeciospores were consistent with published descriptions of *C. woroninii* (Savile 1950; Kuprevich and Tranzschel 1957; Gäumann 1959; Ziller 1974; McBeath 1984; He et al. 1995).

DISCUSSION

This study confirms that spruce is the alternate host of *C. woroninii*. This is supported by the successful artificial inoculation of spruce with basidiospores produced on brooms of *L. groenlandicum* and by the constant field association of infections on both hosts. Although the successful infection was produced outdoors, the chance of two buds becoming infected on one tree from exogenous inoculum originating from distant natural infections is remote. In addition to the control trees kept outdoors with the infected one, there were many mature ornamental spruce of various species in the vicinity. None of these has ever developed spruce bud rust.

This study has also clarified several other aspects of the life cycle of *C. woroninii*. Most notable was the length of time required (nearly 1 year) for systemically infected spruce buds to appear after infection. In nature, infected spruce buds open at the same time as sporulating telia are present on stunted *Ledum* shoots. Therefore, infection must have occurred during the previous year, as suggested earlier by Savile (1950). Such an extended life cycle is different from *Chrysomyxa* species that cause non-systemic needle rusts, in which infection and the production of spermogonia and aecia occur in the same growing season.

Young needles are the most likely rust infection site on spruce, as evidenced by the orange banding of needles during the growing season when infection takes place. Another possibility is that penetration occurs directly into the shoot axis of the newly opened bud; however, infection occurs at a time when overwintered buds have just opened, and very little expansion of this axis has occurred between the needles. In either case, hyphae could grow into or proliferate within the succulent tissue of the expanding shoot tips during the current growing season and then into the winter buds. In spruce, bud scales for the following year are already forming at shoot apices or in the needle axils when shoot elongation occurs during spring and summer. Needle primordia begin to form in these buds as soon as shoot elongation ceases in midsummer, and this continues until buds become dormant in the fall (Heide 1974; Owens et al. 1977), affording ample opportunity for the rust fungus to become established in these buds.

Chrysomyxa woroninii appears to have specific environmental requirements for survival. Its patchy distribution and restriction to subalpine and far northern regions implies a high degree of ecophysiological specialization. In several greenhouse inoculations, the needle discoloration suggested that infection had succeeded. However, lack of further symptom development implied that requirements for dormancy, moisture, or snow cover were possibly not met in the greenhouse. The rust fungus was unable to sporulate normally on the field-collected *Ledum* plant that was overwintered outdoors at Edmonton without snow cover, but it produced telia the second spring, after winter snow cover was provided. These specific environmental requirements might also explain unsuccessful attempts to produce artificial infections with this rust (Klebahn 1914).

Production of aecia on buds adjacent to primary infected buds, as observed by McBeath (1984), was also seen in this study, both in the trees at the Little Berland River site and in a naturally infected tree kept in the greenhouse for one summer. Rather than secondary infections by aeciospores, however, we believe these to be caused by systemic growth of the rust fungus within the spruce shoot as the season progresses. If these 'secondary infections' were caused by aeciospores, one would expect to find newly infected buds on other parts of the tree as well, but this was not observed. The loss of needles for several centimeters below infected buds at the end of the growing season also suggests that mycelium extends for some distance below the infected bud. The rust does not recur on these buds the following year, neither does new growth resume at these bud tips.

Observations of perennial brooms produced by *C. woroninii* on *L. groenlandicum* plants have further clarified its relationship with this host. Once telia have sporulated in the spring, the new leaves that bore them shrivel and fall off. Apart from the stunted shoots, there are no further signs of the rust for the rest of the growing season. The brooms are easily overlooked, especially in areas with a dense shrub layer. Microscopic studies of dormant shoots collected in October confirmed that *C. woroninii* overwinters within the twigs and the young leaves already formed in the dormant buds of brooms.

The relationship of *C. woroninii* to other *Ledum*-infecting rusts needs clarification. In addition to *C. woroninii*, there are at least three other species of *Chrysomyxa* that sporulate on the lower surface of leaves of *L. palustre*, *L. palustre* var. *decumbens*, or *L. groenlandicum* (*C. ledi*, *C. nagodhii* P.E. Crane, and *C. reticulata* P.E. Crane). In addition, *C. ledicola* Lagh. sporulates on the adaxial surface of leaves. On the alternate host, spruce, all of these species produce localized needle infections, but not systemic bud infections, during the same growing season as inoculation (de Bary 1879; Fraser 1911, 1912; Crane et al.1998; Chapter 2). Although Liro (1907) and Jørstad (1934) maintained that aeciospores of *C. ledi* and *C. woroninii* are morphologically similar, the current studies show that they are distinct. For instance, aeciospores of *C. woroninii* are extremely variable in length (up to 62 µm long), they do not have a groove (oriented parallel to the long axis of the spore), and warts are broad and flat-topped. In contrast, aeciospores of European *C. ledi* reach a maximum length of 36 µm, they have a vertical groove, and surface warts are narrow and tapering. The North American hypophyllous *Ledum* rusts also have distinct spore morphology (Crane et al. 1998; Chapter 2). In addition, most of these fungi have a much more widespread habitat distribution than does *C. woroninii* (Jørstad 1934; Ziller 1974; Chapter 2). Their presence on the same hosts probably explains the confusion over whether uredinia occur in *C. woroninii*. Uredinia that appear on leaves of the previous season, of both broomed and healthy *Ledum* shoots, were confirmed by microscopic examination to belong to *C. ledi*, *C. nagodhii*, or *C. ledicola*. Their telia form in small localized groups. Telia of *C. woroninii*, on the other hand, completely cover the underside of systemically infected leaves of the current year.

Where a severe outbreak of *C. ledi* on spruce needles is followed the next year by frequent bud rust infections, this may also lead to confusion (Liro 1907; R. Jalkanen, Finnish Forest Research Institute, personal communication). It is likely that the conditions that are conducive to heavy infections of *C. ledi* in a given year also produce heavy *C. woroninii* infections. However, the outbreak of *C. ledi* would occur the first

year, whereas *C. woroninii* would not appear until the second. This could lead to the conclusion that they are different states of the same rust.

This study has confirmed that *C. woroninii* is a heteroecious rust, with the telia produced on current-year leaves of systemically infected shoots of *Ledum* spp. and the spermogonia and aecia on buds of *Picea* spp.; that it is perennial and systemic in the broadleaved host and systemic and annual in the conifer host; that the bud rust symptom on *Picea* spp. is visible the growing season after the one in which infection occurs; that *C. woroninii* is distinct from other *Chrysomyxa* species that inhabit the same hosts and produce localized infections; and that it has a delicately balanced relationship with both of its hosts. Its ability to coexist with but not seriously damage its host plants, and its requirement for specific environmental conditions to support these relationships, suggest a long history of coevolution among the organisms in this pathosystem.

Fig. 4.1. Witches' broom (arrow) caused by systemic infection of *Ledum groenlandicum* with *Chrysomyxa woroninii*. The other shoots are normal in size. Bar = 2 cm. **Fig. 4.2**. Telia among the hairs on the underside of a newly opened leaf. Arrow points to one pulvinate telium. Bar = 1 mm. **Fig. 4.3**. Longitudinal section of a shoot collected in Oct. 1996 from a witches' broom on *L. groenlandicum*. Note intercellular rust hypha in the cortex of the stem below the bud. Bar = 10 µm. **Fig. 4.4**. Irregularly shaped hyphae in the dense stem tissue immediately below the dormant bud. Bar = 10 µm. **Fig. 4.5**. (*a*) *Picea mariana* artificially inoculated the previous year with basidiospores of *C. woroninii* from *L. groenlandicum*. Arrows indicate two systemically infected buds. Bar = 16 mm. (*b*) Closer view of an infected bud showing stunted needles bearing aecia. Bar = 4 mm.

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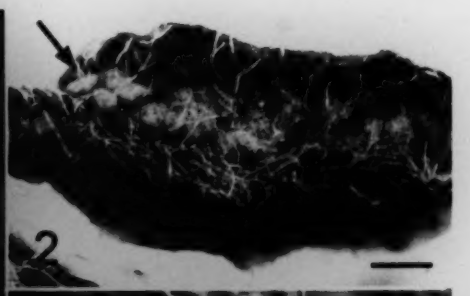
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Chapter 5. Evidence for environmental determination of uredinia and telia production in *Chrysomyxa pirolata* (inland spruce cone rust)[2]

INTRODUCTION

In many rust fungi (Uredinales) the telia are produced in response to environmental conditions that are unfavorable for continued vegetative development of the fungus. The thick-walled teliospores allow the fungus to survive adverse conditions (Waters 1928; Mendgen 1984). In the genus *Chrysomyxa*, however, the teliospores are not overwintering propagules, but form in the early spring and germinate soon after to produce basidiospores. These delicate spores infect young needles or cones of the conifer hosts (mostly *Picea* spp.), where they later form spermogonia and aecia. On the alternate host, the telia are produced before, after, or concurrently with the uredinia in heteroecious macrocyclic species. Although the proportions of these two stages vary on different host plants or within the same host in different geographic regions (Ziller 1974), little attention has been given to the conditions that might favor development of one type of sorus over the other. Because the telia produce spruce-infecting basidiospores, knowing the factors that induce this stage could have economic importance.

Chrysomyxa pirolata Wint. systemically infects cones of several species of *Picea* (spruce), preventing seed formation or resulting in abnormal or reduced seed germination (Nelson and Krebill 1970; Sutherland 1981). Inland spruce cone rust, the disease caused by this fungus, is becoming increasingly important because of the establishment of high-value spruce orchards to produce seed for reforestation in North America and Europe (Sutherland 1990). The rust fungus is heteroecious, with alternate hosts in *Pyrola* and *Moneses* (Ericaceae, Pyroloideae), small evergreen understory plants that are common throughout the boreal forests of the northern hemisphere. Although a few attempts have been made to control the rust chemically (Summers et al. 1986; Annila and Heliövaara 1991), these efforts have been hampered by a lack of knowledge about certain aspects of

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the life cycle of *C. pirolata*. For example, there is great variability in the proportion of uredinia and telia produced among plants of the same *Pyrola* species (Summers et al. 1986). In some locations telia are rare or absent (Wilson and Henderson 1966). An understanding of this variability might lead to a greater understanding of the variation in cone rust levels from year to year (Fraser 1912; Ziller 1974; Singh and Carew 1990).

At the Alberta Tree Improvement and Seed Centre, Smoky Lake, in central Alberta, cone rust levels have varied from 0 to 25% of cones in recent years. This nursery is surrounded by natural spruce forest (*Picea mariana* (Mill.) B.S.P. and *P. glauca* (Moench) Voss) containing alternate hosts (mainly *Pyrola asarifolia* Michx.) of the cone rust, and it was chosen as the primary site for this study. Infected *P. asarifolia* plants were studied at this location and at two others in central Alberta over 3 years. The main objectives of this study were to observe the yearly disease cycle of the rust and to test the hypothesis that moisture levels affect the formation of telia in *C. pirolata*.

MATERIALS AND METHODS

Field observations

In September 1996, *Pyrola asarifolia* plants were located in natural forests adjacent to two white spruce (*Picea glauca*) orchards that were part of a tree improvement center. The *P. asarifolia* plants were examined for signs of *C. pirolata* infection because the orchard trees had been infected with *C. pirolata* that year. One natural site (W) was low-lying and boggy and dominated by *Populus balsamifera* L., with an understory of *Ledum groenlandicum* Oeder, *Rosa* spp., and *Caltha palustris* L. The other site (D, 6 km NW of site W) was drier, higher elevation, and dominated by *Populus tremuloides* Michx., *Picea glauca*, and *Pinus banksiana* Lamb. with an understory of *Alnus crispa* (Ait.) Pursh, *Prunus virginiana* L., *Amelanchier alnifolia* Nutt., *Rosa* spp., and *Arctostaphylos uva-ursi* (L.) Spreng. The following spring (on May 1) individual infected *P. asarifolia* plants, identifiable by the presence of orange gelatinous sori on the leaves, were tagged and numbered for identification, 13 plants at site D and 5 at W. The tagged plants were examined weekly during May and early June for disease progression

and thereafter biweekly until the end of July. Percentage of the leaf surface covered by uredinia and telia was estimated for each plant with the aid of a hand lens. Uredinia could be identified by the presence of powdery urediniospores, and telia by their paler yellow color and fuzzy appearance due to basidia on the surface. Weather data (rainfall, maximum and minimum relative humidity, and maximum and minimum temperature), collected about 1 km from W, were obtained from the Tree Improvement Centre for May, 1997 and 1998, and were compared with the timing of sorus maturation for both years.

In May 1998, infected plants were again observed at the two sites for the proportions of uredinia and telia. Although most of these were the same tagged plants as the previous year, some plants had died or had been removed and dissected to determine the location of hyphae; therefore others were added to make up 13 plants at each site: at W, 4 of the original 5 plants were available; at D, 9 of the original 13 plants were available.

Controlled-environment studies of telia production in the laboratory

To test the hypothesis that moisture is a determining factor in the production of telia, controlled-environment experiments were done in May 1998. Infected *P. asarifolia* plants were collected from three locations in central Alberta from Apr 28 to May 12, 1998: Edmonton, Smoky Lake (site D, described above), and 27 km S of Hinton. The plants had leaves either completely covered with undifferentiated sori or they had some uredinia (usually at the leaf edges) and the rest undifferentiated. None of the experimental plants had telia. In all, 23 plants were placed under high humidity (90–100%), day lengths of 0 or 15 h, and various day/night temperatures (details, Table 5.1). In about one-third of the plants, a water-soluble marker was used to mark the position of uredinia at the start of the experiment; other plants were photographed before and after experiments. Experimental plants (aboveground parts plus part of the rhizome and roots) were either placed in sealed petri dishes containing moist filter paper; potted in 12-cm plastic pots with surrounding soil, then watered, misted, and covered with a plastic bag to maintain 100% humidity; or potted and kept in a controlled-humidity incubator.

Table 5.1. Summary of the artificial conditions used to test the effect of high humidity on the development of telia in systemically infected *Pyrola asarifolia*

Expt. no.	Conditions[a]	Source of infected plants	No. of plants
1	15 h, 16/6, 100	Edmonton	3
2	15 h, 23/6, 100	Smoky Lake	3
3	15 h, 23/6, 90	Smoky Lake	4
4	0 h, 22/22, 100	S. of Hinton	4
5	0 h, 4-6/4-6, 100	S. of Hinton	3
6	15 h, 16/4, 100	S. of Hinton	3
7	15 h, 8/2, 100	S. of Hinton	3

[a]Conditions list day length, h; day/night temp., °C; humidity, %.

Controlled-environment studies of telia production in the field

The Hinton site was chosen for a field experiment of telia formation in 1999. The slight south-facing slope and dry soil conditions, as well as the high production of uredinia the previous year, made it the most suitable site to test artificial induction of telia. Unfortunately, the weather during May 1999 was much cooler and wetter than during May the previous year, potentially producing ambiguous results. On 23 May 1999, 36 systemically infected plants (identified by crowded sori covering undersides of leaves) were tagged at 12 different microsites at this location. Sixteen of these already had some visible uredinia; the rest of the sori were either subepidermal or barely erumpent through the epidermis. At each microsite, one or more plants were sprayed with water and covered by a clear plastic bag that had two small holes for ventilation, and the bag was anchored by small stakes. One or more nearby infected plants with sori at a similar stage of maturity were not watered or covered and were marked as controls. They were within 50 cm of covered plants. After 1 week, the bags were removed and all plants harvested and taken to the laboratory for comparison of sorus development between test plants and nearby controls. They were renumbered by another person before examination to avoid bias in determining the percentage of each sorus type on controls and covered plants. Each leaf of each plant was examined separately and a visual estimate of the percentage area covered with each type of sorus was obtained. When sori could not be identified as either uredinia or telia, but were quite swollen, one or more strips of leaf tissue (about 2 × 4 mm) bearing representative sori were removed from each leaf with a razor blade. These were sectioned by hand with a razor blade and examined by light microscope. Plants with insufficient development of the fungus for sorus identification were discarded.

Light and scanning electron microscope studies of sori of C. pirolata

Cross sections of infected leaves of *P. asarifolia* from both experimental and field-collected plants were made by hand to compare the morphology of uredinia, telia, and undeveloped sori. Sections were mounted in lactophenol for examination by LM.

For SEM, segments of sorus-bearing leaf tissue (3–4 mm square) were washed in phosphate buffer (pH 7.0) and fixed in 3% glutaraldehyde in buffer for 2–4 h at 5°C. They were rinsed with buffer, then immersed in 2% tannic acid – 2% guanidine hydrochloride solution for 4 h or longer at 5°C. The leaf tissue was then washed in distilled water and postfixed overnight in 2% OsO_4 at 5°C. The fixed material was dehydrated in an ethanol series, taken to amylacetate, and critical point dried using carbon dioxide. The dried samples were coated with gold and examined with an Hitachi S-510 scanning electron microscope operated at 15 kV.

RESULTS

Disease cycle in Pyrola and natural production of telia at two sites at Smoky Lake

In early 1997 there was heavy snow cover during the winter and the ground was still frozen on May 1 at the wet site. Standing water was common. Sori on *P. asarifolia* became swollen sooner on the wet site than on the dry one. By May 15, at the wet site, differentiation of telia had begun on four of the five plants; the fifth plant was on a grassy hummock, and leaves bore 100% uredinia. At the dry site, no telia were found on May 15, but many leaves had uredinia. After rain fell on May 16, all previously undifferentiated sori at both sites became telia. The final proportions of telia to uredinia varied from plant to plant and even from leaf to leaf in the same plant.

In spring 1998, although there was less standing water at the wet site than in 1997, the soil was still saturated with moisture. After a mild winter and exceptionally warm spring, development of uredinia had already begun by May 1 at both sites. Uredinia appeared first at the edges and base of leaves. Unlike 1997, almost all plants at the wet site had some uredinia by May 7, and at the dry site a few plants had nearly 100% uredinia by this date. Such plants were always in open sunny microsites. At the same time, all 13 plants at the wet site also bore 10 to 80% telia on their leaf surfaces, whereas only 4 of the 13 plants at the dry site had begun telia differentiation. The latter were in low-lying shaded microsites. At the next observation date (May 14) only telia development was occurring at both sites. Although little rain fell (total of 4.8 mm) during

the entire month of May at Smoky Lake, the maximum daily relative humidity increased after May 9 to 60–99%; it had been 30–62% earlier in the month. The rise in maximum daily relative humidity corresponded to low minimum daily temperatures and likely dew formation.

At the dry site, telia predominated (>50% of lower leaf surface) on 31% of plants in 1997 and on 54% in 1998. At the wet site, telia predominated on 80 and 85% of the infected plants in 1997 and 1998, respectively (Fig. 5.1).

Overwintered leaves with only telia often appeared wilted and were often flipped over, exposing the sporulating surface. Leaves died after sporulation of the rust, but those bearing mostly uredinia survived longer than those bearing mostly telia. On leaves of the current season, telia were never observed, and uredinia were rare. In fact, after the death of the old leaves, infected plants were difficult to detect, although some were slightly chlorotic or had a more upright habit than healthy plants. When systemically infected plants were dissected, rust hyphae could be seen in the leaves, petioles, buds, and rhizomes. They were mostly intercellular within the cortex of petioles and rhizomes, 6 – 8 µm wide, and they produced simple vesicular haustoria in the cells. Occasionally a few localized uredinia were seen in early spring on otherwise healthy plants adjacent to systemically infected ones. These may have been new infections initiated by urediniospores or aeciospores the previous year.

In 1998, at some locations at both sites, there was a large increase in systemically infected plants close to tagged plants from 1997. Several of the marked plants could not be found during the second spring.

Controlled-environment studies of telia production in the laboratory

With the exception of four experimental plants, sori (Fig. 5.2) differentiated into only telia when they were placed under high humidity in petri dishes or an incubator. This happened regardless of temperature or light conditions and in most cases regardless of whether uredinial production had begun at the leaf edges before the experiment. Low temperature, however, slowed the formation of telia from sori. For example, at 22°C and

100% relative humidity, telia reached maturity in 3 days, whereas this process took 6 days at 4–6°C. Two plants that had a large proportion of uredinia at the start of the experiment continued to develop only uredinia. Two other plants had very immature sori at the beginning of the experiments, i.e., they were small and not prominent, and the rust did not continue development under the stress of transplanting. Within each experiment, there was some variability among plants in the time required for telia development, depending on the initial maturity of the sori.

Difficulties were encountered in the use of field controls to compare with experimental plants in the laboratory experiments. Initially we planned to place naturally infected plants under both dry and moist conditions and then to compare the proportions of uredinia and telia produced. However, when infected plants were placed in pots under low humidity, even if soil was kept wet, the infected leaves died from the stress of transplanting. When plants were placed under high humidity, the systemically infected leaves usually survived and the rust continued developing. Likewise we tried to use tagged, infected plants that were near the source of the infected experimental ones in the field as controls. It was not possible to control the environmental conditions surrounding these plants, and in some cases the controls died because of removal of experimental plants that had belonged to the same clone. Sori on field-control plants were also much slower to develop than on experimental plants, making comparison difficult (Fig. 5.3). However, the abrupt change in differentiation of sori from uredinia to telia when placed under high humidity is convincing evidence that this is an important factor in telia production (Figs. 5.4–5.6).

The plants from the Hinton site produced the most conclusive results because of the overwhelming production of uredinia when they were collected. No telia were found at this dry site at the collection date. Of 25 infected plants, 12 already had 100% uredinia. The 13 plants used in the moisture experiments had either 100% undeveloped sori or a mixture of uredinia and undeveloped sori. During the moisture experiments all undeveloped sori differentiated exclusively into telia. Photographs taken before and after the experiments (Figs. 5.4, 5.5) and marker lines surrounding the uredinia at the

beginning of the experiments (Fig. 5.6) confirmed the abrupt change from differentiation of uredinia to telia under high humidity.

Controlled-environment studies of telia production in the field

The proportions of telia, uredinia, and undeveloped sori on control and covered plants from the Hinton field experiment are given in Table 5.2. Only four of the plants had mature (sporulating) telia after 1 week. These were all on covered plants. Of the seven usable sets of control and covered plants (total = 19), two had all or mostly uredinia on both control and covered plants. Three showed much greater production of telia in the covered plants, and two showed only a slightly greater proportion of telia in the test than in the control plants.

Morphology of sori

In cross section, telia and uredinia were distinct morphologically. Mature uredinia were cup-like structures with a rudimentary peridium and contained chains of ornamented urediniospores and intercalary cells produced from the base of the sorus. Telia, on the other hand, had a constricted base made up of chains of narrow, elongated, thin-walled, colorless sterile cells; the more rounded, thin-walled orange-colored teliospores were borne in chains distal to the sterile cells towards the surface of the sorus. Young telia had an amorphous material covering the outer surface. When mature, the teliospores germinated to produce curved, four-celled basidia. Undeveloped sori had a disorganized appearance and the cells could not be distinguished as either urediniospores or teliospores. Both light and scanning electron microscopic examination revealed that young uredinia sometimes converted to telia. These sori usually occurred at the transition area between groups of uredinia and telia. Urediniospores formed in the center of the sorus, while basidia arose around the outside of the sorus (Fig. 5.7, A–C).

Table 5.2. Comparison of sorus development in infected *P. asarifolia* plants covered with plastic bags for 1 week with nearby control plants left uncovered, S of Hinton, May 1999

Microsite	Plant	Leaf	Uredinia, %	Telia, %	Undeveloped[a]
A	Covered	1	98	–	2
	Control	1	95	--	5
B	Covered	1 – 4	5 – 10	5 – 10	85 – 90 (III)
	Control 1	1	50	–	50 (III)
		2	90	–	10 (III)
		3	80	–	10 (III)
		4	98	–	2 (III)
		5	40	–	60 (III)
	Control 2	1-6	80 – 90	–	10 – 20 (III)
C	Covered	1	2	98	--
	Control	1	100	--	0 (III)
		2	30	--	70 (III)
		3	20	--	80 (III)
D	Covered	1 – 2	100	--	--
	Control	1 – 2	80	--	20 (II)
E	Covered	1	20	10	70 (III)
		2	80	–	20 (III)
		3	5	2	93 (III)
		4	5	–	95 (III)
	Control 1	1	100	–	--
	Control 2	1 – 2	5	--	95 (III)
		3	50	--	50 (III)
		4 – 5	60	--	40 (III)

Microsite	Plant	Leaf	Uredinia, %	Telia, %	Undeveloped[a]
F	Covered 1	1	15	85	--
		2	20	80	--
		3	40	60	--
	Covered 2	1	5	95	--
		2	20	80	--
	Control	1	98	--	2
		2	90	--	10
G	Covered	1 – 6	0 – 1	--	99 – 100 (III)
	Control1	1 – 2	100	--	--
	Control 2	1	100	--	--
	Control 3	1 – 2	100	--	--

[a]Roman numeral after percentage of undeveloped sori indicates the stage that was forming as determined by microscopic examination of sections of sori; II = uredinia, III = telia.

DISCUSSION

Sori of *C. pirolata* produced on overwintered leaves of *P. asarifolia* have the potential to become either uredinia or telia, and their differentiation and development is influenced by environmental conditions. These hypotheses are supported by field observations of greater proportions of telia on plants in moist microsites, an increase in telia production associated with high daily maximum relative humidity and rainfall, the change from production of uredinia to production of telia when plants were subjected to high humidity in the laboratory and in the field, and microscopic observations of sori transforming from uredinia to telia. Photographs of plants taken before and after the laboratory experiments, and marks on leaves delineating the location of uredinia show that the change from production of uredinia to telia was abrupt. It might be argued that the direction of sorus development is predetermined and that telia simply develop later than uredinia. Although my observations do suggest that telia tend to develop later in the spring than uredinia, the observed variability in maturity of sori at a given site and among sites makes it unlikely that I happened to choose plants that had already ceased uredinial production before the experiments. It might also be argued that disturbance to the root systems by potting of the plants could have caused the change from one form of sorus to the other. However, the different proportions of the two stages at the wet and dry sites at Smoky Lake under natural conditions support the involvement of moisture in differentiation.

This is believed to be the first attempt to explain the variability in production of uredinia and telia among different individuals of *P. asarifolia*. There have been few reports of the factors that induce telia in rust fungi. Most studies have concerned rusts on horticultural and cereal crops that produce telia late in the growing season as a means of overwinter survival. During this process, old uredinia may be converted to telia (Rothman 1974). Telia formation of some of these rusts can be induced by unfavorable conditions, such as low temperature in darkness (Waters 1928; Yeh et al. 1981) or by the presence of hyphomycetes such as *Aphanocladium album* (Preuss) W. Gams and *Septoria nodorum* Berk. (Biali et al. 1972; van der Wal et al. 1970). Differentiation may also

depend on a particular combination of rust isolate and host variety (Jackson and Young 1967; Rothman 1974; Dufresne et al. 1987). In white pine blister rust (*Cronartium ribicola* J.C. Fischer), cool temperatures seem to be a requirement for induction of telia (Riker et al. 1947). The physiological mechanism of teliospore formation is likely different from that of *C. pirolata*, in which telia are not winter survival structures. The thin-walled teliospores of *C. pirolata* form in spring and they germinate almost immediately.

Microscopic observations of uredinia converting to telia at the transition zone between groups of the two types of sorus also suggest a degree of plasticity in the direction of sorus differentiation. Because sorus development occurs from dividing cells at the base of the sorus, and sterile cells characteristic of telia were forming beneath the uredinial hymenium, I concluded that the direction of change was from uredinia to telia and not the opposite. However, in both the field and the laboratory experiments, a few plants continued to develop only uredinia in spite of the experimental conditions. All of these plants had a large proportion of differentiated uredinia before the experiments. Once sori reach a certain maturity, their development may not be reversible.

Observations in both the field and laboratory indicate that temperature is also an important factor in the maturing of sori, and they remain dormant at a few degrees above 0°C. The only mature, sporulating telia found at the end of the field experiment at Hinton were on leaves of covered plants; although small holes were made in the bags to allow ventilation, this accelerated development may suggest that there was a higher temperature under the bags than in the surrounding atmosphere.

The suggestion that moisture is a determining factor in telia differentiation in *C. pirolata* may explain the variability in cone rust incidence from year to year (e.g. 0–25% adjacent to site D at Smoky Lake) and may also have important implications for the placement of spruce seed orchards. Sori on *Pyrola* plants in sunny dry spots tend to differentiate early, and to form uredinia, which when mature do not appear to be able to convert to telia. Plants in moist, low-lying or boggy sites tend to develop slowly and to form telia regardless of weather conditions, as shown by the predominance of telia at site

W even during an exceptionally dry spring. *Pyrola* plants at such sites may therefore pose a greater threat to nearby spruce cones because their chances of having undifferentiated sori when rainy weather occurs is greater. The possibility that infected *Pyrola* plants in open dry forest are less likely to cause cone disease in adjacent spruce than are plants in dense, low-lying forest needs to be further investigated.

The production of telia only under specific environmental conditions has adaptive significance. They form only when there is moisture to promote germination or when there is less chance of damage to the delicate basidiospores by desiccation or ultraviolet radiation. The coincidence of basidiospore dissemination with periods of precipitation or at night has been observed in other rust fungi (Van Arsdel 1967; Mazzola and Bergdahl 1989), but this report of actual formation of telia being governed by environmental conditions appears to be unique. When conditions are inappropriate for telia formation (and probably also the survival and germination of basidiospores), the fungus maximizes production of uredinia and expends energy in building up infections asexually in the *Pyrola* hosts. The possibility that other rust species also form their telia in response to specific environmental conditions needs to be examined.

Several other aspects of the life cycle of *C. pirolata* in *P. asarifolia* were clarified during this study. Observation of the same plants over two growing seasons confirmed the perennial nature of this rust. The production of telia and most uredinia is confined to systemically infected leaves from the previous year and occurs during a very limited time in early spring. Overwintered leaves die after sporulation of the rust, whether uredinia, telia, or a combination of both are produced. The observation that leaves with telia die sooner than those with uredinia may suggest that formation of telia exerts greater stress on the leaves, perhaps by their moisture or energy requirements. Uredinia were rarely observed on current-year leaves, even late in the growing season. New infections are likely difficult to observe, because they may not sporulate until the next year.

This paper presents new basic biological information on the life cycle of *C. pirolata*. This information is relevant to the management, prevention, and control of spruce cone rust damage in high-value spruce seed orchards and cone-collecting areas.

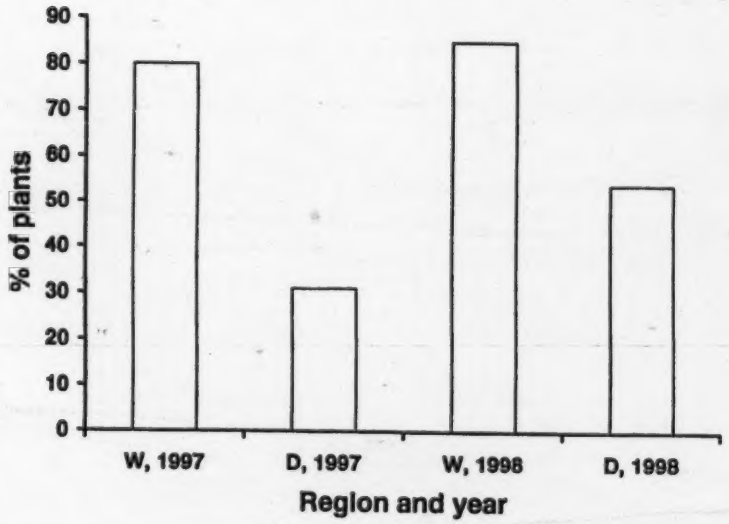
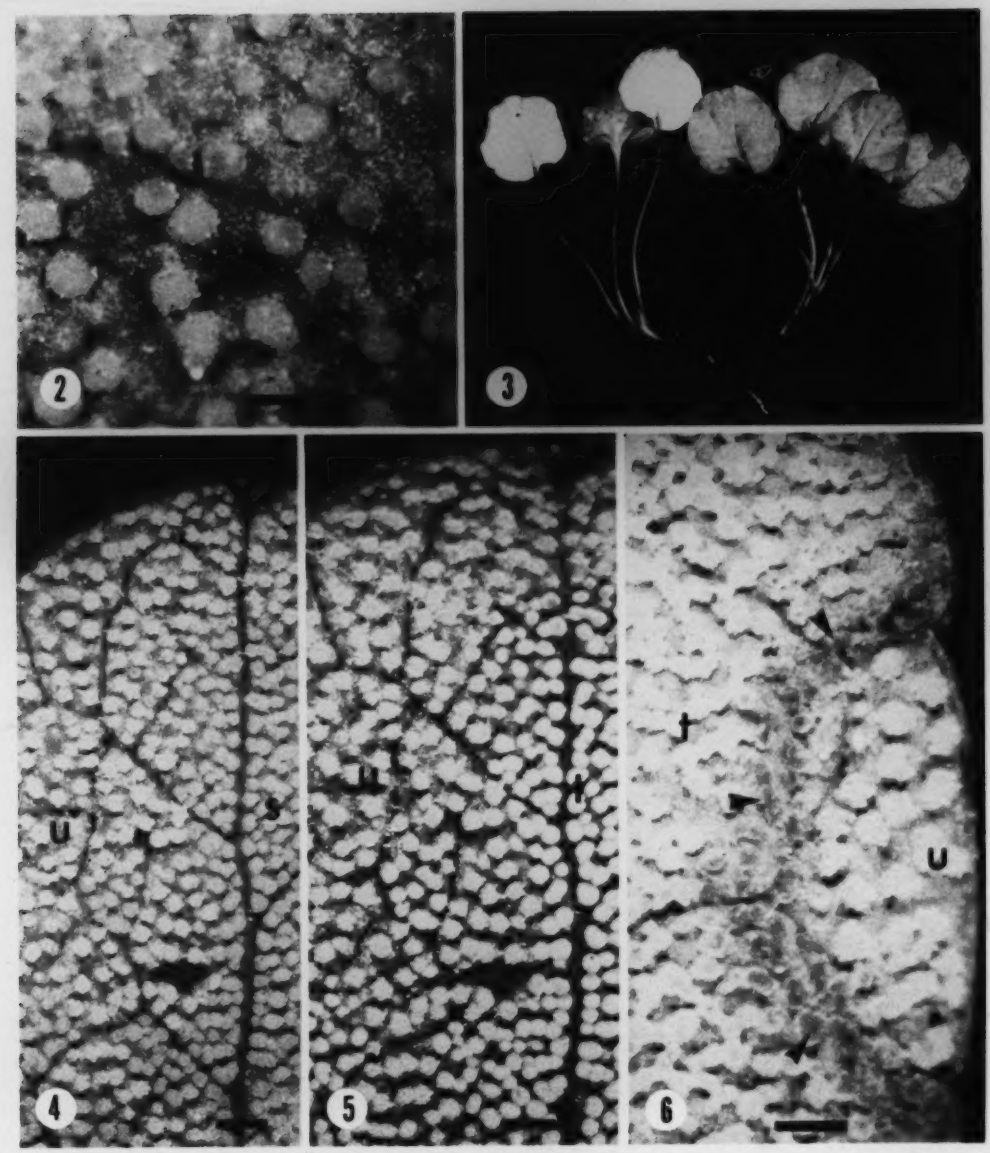


Fig. 5.1. Percentage of *P. asarifolia* plants infected with *C. pirolata* that had greater than 50% of their leaf surface covered with telia in 1997 and 1998 at a dry site (D) and a wet site (W). In each case, 13 plants were examined, except for W in 1997, for which only 5 infected plants were found.

Figs. 5.2–5.6. Results of experiments to determine the effect of moisture on the development of telia of *C. pirolata* in *P. asarifolia*. **Fig. 5.2.** Prominent gelatinous sori on abaxial leaf surface; they have not yet differentiated into uredinia or telia. **Fig. 5.3.** Left, plant with only telia, artificially induced in an incubator (6 days at 100% relative humidity, 15-h day, 16°C day and 6°C night) and, right, an adjacent plant left under field conditions for the same length of time; the latter plant has a few uredinia, but mostly undeveloped sori. About one-half natural size. **Fig. 5.4**. Leaf before experiment, with powdery uredinia (*u*) at edges and undeveloped sori (*s*) at the center, around the main vein. **Fig. 5.5**. Same leaf as in Fig. 5.4, after 6 days in a moist chamber in a refrigerator. Uredinia (*u*) are more open, and sori in the leaf center have become fuzzy telia (*t*). **Fig. 5.6.** Leaf with a line (arrows) showing location of uredinia (*u*) at edge of leaf before the experiment, and adjacent sori that became telia (*t*) during 7 days under 90% humidity, 16°C day and 6°C night temperatures, and 15-h day length. The telia become confluent as they age. In Figs. 5.2, 5.4–5.6, scale bars = 1 mm.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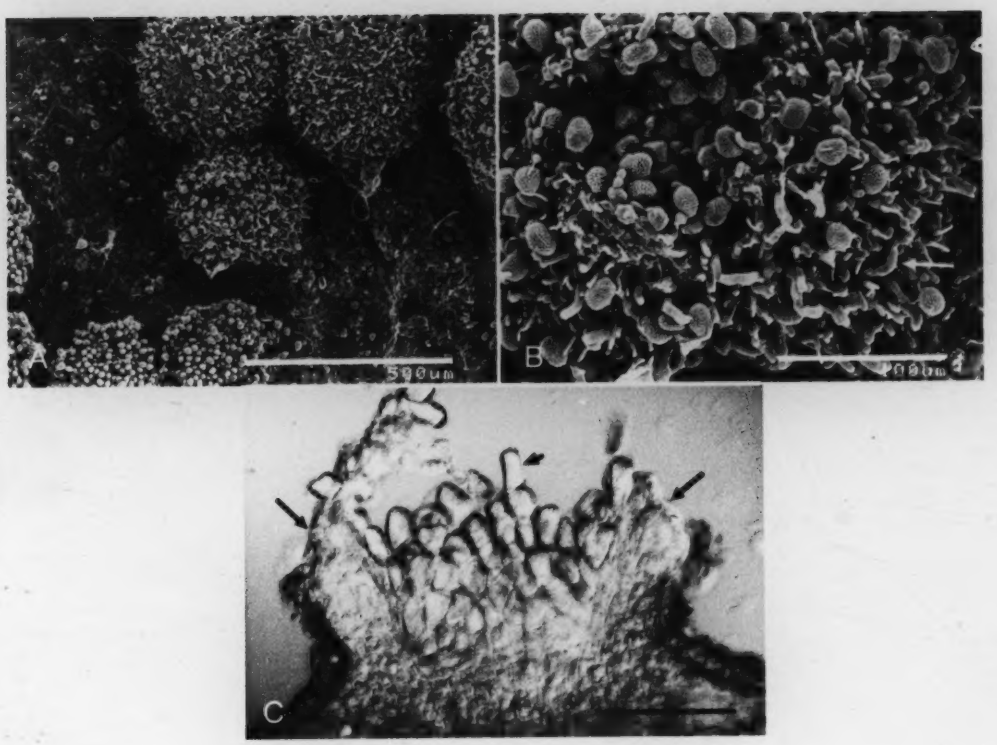
6

Fig. 5.7. Sori of *C. pirolata* on *P. asarifolia* leaves. (A) Uredinia (short arrow), uredinium converting to a telium (center), and larger telia (long arrow). Scale bar = 500 μm. (B) Close-up of transforming uredinium from (A). Ornamented urediniospores are forming in the center of the sorus, basidia around the outside (arrow). Scale bar = 100 μm. (C) Cross section of a transforming sorus similar to that in (B), with urediniospores in center (short arrow) and teliospores at the sides (long arrows). Closely compact rectangular cells below the urediniospores more closely resemble the cells at the base of a telium rather than the more rounded cells of a uredinium. Scale bar = 25 μm.


A
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B
C

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Chapter 6. Reproductive biology and evidence for water dispersal of teliospores in *Chrysomyxa weirii*, a microcyclic spruce needle rust[3]

INTRODUCTION

Among the North American species of the genus *Chrysomyxa*, *C. weirii* Jacks. is unique in being autoecious and microcyclic. The rust fungus forms reddish-orange, tonguelike telia in early spring on spruce needles of the previous year, and the infection spreads to young needles on newly opened buds before the old infected needles drop off. The natural host range for *C. weirii* includes *Picea engelmannii* Parry, *P. glauca* (Moench) Voss, *P. mariana* (Mill.) B.S.P., *P. rubens* Sarg., and *P. sitchensis* (Bong.) Carr. (Arthur 1934, Savile 1950, Ziller 1974). Although its occurrence is more sporadic than most other spruce needle rusts in boreal and subalpine forests (Peterson 1961), it has an extensive range that includes northern Oregon, Idaho, western Montana, Washington, South Dakota, British Columbia, Alberta, Saskatchewan, Manitoba, New Brunswick, Northwest Territories, Yukon Territory, as well as the southern Appalachian Mountains (Weir 1923, Boyce 1943, Peterson 1961, Ziller 1974). In recent years *C. weirii* has been introduced to blue spruce (*P. pungens* Engelm.) nurseries in the northeastern United States (Vermont, New Hampshire, Pennsylvania) (Pawuk 1971, Bergdahl and Smeltzer 1983, Merrill et al. 1993), where serious outbreaks affecting up to 100% of new needles on the lower branches have been recorded (Bergdahl and Smeltzer 1983). There is also one report of *C. weirii* on *P. schrenkiana* Fischer & Meyer from south-central Asia (Kuprevich and Tranzschel 1957).

As in other members of the genus *Chrysomyxa*, the teliospores of *C. weirii* are thin-walled and catenulate, and they germinate without a period of dormancy. However, this single stage provides few systematic characters for comparison with other *Chrysomyxa* species, most of which are heteroecious and macrocyclic, with their telia on various genera of Ericaceae. Therefore a study of the entire life cycle of *C. weirii* was

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[3]A version of this chapter has been published: Crane, P.E., Hiratsuka, Y., and Currah, R.S. 2000. Mycologia, 92: 754–763.

undertaken in a search for characters that might elucidate its relationship with other members of the genus. In this study I describe the cytology and the unique form of teliospore germination in *C. weirii*, and discuss the possible implications of these features for its dispersal.

MATERIALS AND METHODS

Specimens of *C. weirii* were examined from the Mycological Herbarium of the Northern Forestry Centre (CFB), Edmonton, Alberta, and the Pacific Forestry Centre (DAVFP), Victoria, British Columbia, Canadian Forest Service; and the Arthur Herbarium (PUR), Purdue University, Lafayette, Indiana. The following collections were examined (host, location, collection number): *P. engelmannii*, Alberta, CFB 7967, British Columbia, CFB 458, CFB 7960, CFB 22109 (ex DAVFP), CFB 22111 (ex DAVFP 69-9-0775-01), Idaho, PUR 4906, Oregon, PUR 4907 (Type); *P. glauca*, Alberta, CFB 4903, CFB 5742, CFB 8089, CFB 20015, CFB 22077, CFB 22195, CFB 22196, CFB 22198, British Columbia, CFB 8498, Saskatchewan, CFB 20816, Yukon Territory, CFB 7495, CFB 8904; *P. mariana*, Manitoba, CFB 22175; *P. pungens*, New York, PUR 49269; *P. rubens*, West Virginia, PUR 44396; *P. sitchensis*, Oregon, CFB 8818. Three fresh collections, one from Kananaskis (CFB 22198) and two from Jasper (CFB 22195, CFB 22196), Alberta, were used to study the cytology and spore germination. Morphology was studied by light microscopy using squash mounts of telia and cross sections of spruce needles bearing sori mounted in lactophenol or lactophenol – cotton blue.

Teliospore germination—Spruce needles bearing mature telia were attached with petroleum jelly to the lids of petri dishes containing moist filter paper. Glass slides were placed below the needles to capture basidiospores, and the dishes were sealed and kept in a refrigerator (4 C) or at room temperature (20 C) for up to 1 wk. Teliospores were also dispersed in droplets of water on glass slides and kept in a moist chamber. They were examined by light microscopy at regular intervals from 2 h to 3 d after dispersal. Because initial studies showed some variation in type of germination, slides were kept in

a refrigerator in darkness or at room temperature either under fluorescent light or in darkness, and the results were compared.

Cytological studies—The fluorescent dye 4',6-diamidino-2-phenylindole (DAPI) was used to study the nuclear condition of the various stages in the life cycle of *C. weirii*: hyphae in cross sections of infected needles, squash mounts of the base of telia, ungerminated teliospores, germinating teliospores (at 2, 3, 4.5, and 22 h after dispersal of teliospores in water), and basidiospores. DAPI (0.2 μg/mL) was dissolved in McIlvaine's buffer (0.1 M citric acid:0.2 M dibasic sodium phosphate, 1:6.7) (pH 7.0) (Meixner and Bresinsky 1988). The fresh stain was applied to the fungal material on glass slides, which were viewed immediately under epifluorescent illumination using a Zeiss Axiophot Photomicroscope. In some cases slides of germinating teliospores were dried on a slide warmer (55 C) and stained later (Imazu et al. 1989). To verify the results with DAPI, portions of spruce needles bearing telia were also stained with hematoxylin (Sass 1958). Tissue was first fixed in formalin – acetic acid – ethanol for 3 d, then rinsed in 50% ethanol, dehydrated in a *n*-butanol series, and embedded in paraffin (Sass 1958). Sections (10 μm thick) were made using a rotary microtome and mounted on glass slides with Haupt's adhesive (Gurr 1965). Slides were dewaxed in xylene, rehydrated in an ethanol series, placed in 4% iron alum mordant for 4 h, rinsed in distilled water, and stained in Heidenhain's hematoxylin for 4 h, then destained in diluted iron alum as necessary (Sass 1958). Slides were dehydrated in an ethanol series then placed in xylene before coverslips were permanently mounted with Permount (Fisher Scientific Co.).

Microscopy of inoculated spruce—To study spore germination on host tissue, newly opened vegetative buds of 3-yr-old greenhouse-grown *P. glauca* were inoculated in two ways. Needles bearing mature sori were attached to the lids of petri dishes above excised shoots in a moist chamber, as above. In other cases, sori were wetted with droplets of distilled water, then telia were rubbed along the young needles, and the shoots were kept in a moist chamber either at room temperature or in a refrigerator. At regular intervals from 5 to 30 h after inoculation, thin segments of the upper surface (epidermis and part of underlying mesophyll) of individual needles were removed with a razor blade,

mounted in lactophenol – cotton blue, and examined by light microscopy for spore germination on the host surface. In fresh collections of infected spruce, young current-year needles adjacent to sporulating needles were examined for evidence of natural spore deposition and germination. Host epidermis was removed and examined microscopically in the same manner as for artificially inoculated needles. For scanning electron microscopy (SEM), young current-year spruce shoots, both artificially and naturally inoculated as above, were vapor-fixed in OsO_4: shoots were enclosed in sealed petri dishes containing 3 or 4 drops of 2% OsO_4 in phosphate buffer, pH 7, for 24 h. They were then either air-dried or frozen in liquid nitrogen and freeze-dried, coated with gold using a sputter coater, and examined with an Hitachi S-510 scanning electron microscope operated at 15 kV.

Light microscope images were made by digitizing black and white photographic negatives made from Kodak TMAX 100 or 400 film. SEM images were captured using Quartz PCI software, version 4.0. Image contrast was adjusted and all halftone plates were composed using Adobe Photoshop 5.0.

RESULTS

Morphology

Telia of *C. weirii* occur on well-defined chlorotic bands on second-year needles; they are about 1/3 to 2 mm long, amphigenous, and seldom confluent. Within the needle tissue, the telium is bounded by a layer of small pseudo-parenchymatous cells at the base, and several layers of much larger, thin-walled cells at the sides (Fig. 6.1, A). The teliospores are produced in narrow chains from the basal cells and are extruded between flaps of the host epidermis to form a waxy, reddish-orange, tonguelike mass. The mature spores separate readily, and crusts of spore masses are common on the needle surface around the sori (Fig. 6.1, B). The teliospores are extremely variable in shape, from irregular to ellipsoidal, cylindrical, or rhomboidal with ends obtuse, attenuate, or truncate (Fig. 6.2); the width covers a narrow range from 5 to 9 µm, but the length is variable, 14 – 36 (– 42) µm. The wall is thin (0.4 – 0.8 µm) and colorless. The proximal ends of the

spores have one or two hyaline tails that are either pointed or truncated and about 1.5 – 3.5 μm long. On the distal end of mature spores there is a narrow extension of the cytoplasm within the wall, suggesting a pore, and at this point a globose, knoblike process often extends beyond the end of the mature spore (Fig. 6.2). The knob expands to become the basidium during teliospore germination. Neither the tails nor the basidial knobs were included in teliospore measurements. Small globose, subglobose, or irregularly shaped spores about 5 – 8 × 5 – 7 μm were often seen among the teliospores in squash mounts from the sorus or from the spore crusts taken from the needle surface. These seldom had an apiculus. Empty teliospores, sometimes with a basidium forming from one end, were also occasionally present in these mounts. Although some basidia had one septum, sterigmata were not seen, and basidiospores were infrequent.

Teliospore germination and basidiospore formation

Attempts to capture basidiospores beneath mature telia in a moist chamber were unsuccessful. However, when telia were touched to a droplet of water on a glass slide the spore mass disintegrated and teliospores dispersed readily into the water and germinated. When dispersed in water, the spores remained suspended or sank to the bottom. The most common type of germination is shown in Figs. 6.3, A–D. Shortly after dispersal of teliospores in water (2 h at room temperature), the knoblike processes on the spores expanded into elongated promycelia (basidia) into which the spore contents moved. By 3 h, a single septum had formed across many basidia, and one sterigma (3 – 4 μm long) had grown from each basidial cell. As the basidiospore formed at the end of each sterigma, the cytoplasm moved from the basidial cell into the basidiospore. The distal cell of the basidium usually formed a sterigma and basidiospore earlier than the proximal cell. Basidiospores were globose to subglobose (5 – 6 × 5 – 6 μm) with a small apiculus; they often germinated on the slides, sometimes while still attached to the sterigma, to produce a short sterigma and secondary spore or a short germ tube (Fig. 6.4). In many cases, basidia became septate but failed to develop further to produce sterigmata or basidiospores even after 24 h (Fig. 6.5, A). In one sample of teliospores on a glass slide

for 3 d, many of the cells of the basidia had become rounded and the distal cell had produced a short germ tube, sometimes with a swollen end.

The mode of germination varied with the conditions under which the slides were incubated. At 4 C the two-celled basidium with basidiospores was produced almost exclusively. At room temperature, about one-half of the teliospores formed two-celled basidia and basidiospores (Fig. 6.3), whether in light or darkness. The following types of germination were also observed, more commonly at room temperature: (i) a narrow, greatly elongated, septate or aseptate hyphalike promycelium, with or without a swollen sporelike end, developed from one end of the teliospore (Fig. 6.5, B); (ii) the basidium was elongate, remained aseptate and formed a narrow germ tube from the distal end (Fig. 6.5, C) or a single, unusually long, tapered sterigma (Fig. 6.5, D); (iii) the basidium consisted of a single small cell that formed a more typical short sterigma; (iv) a single sterigma with a basidiospore formed directly from the end of the teliospore; (v) two or more septa formed in the basidium, but the basal cells were empty and only the two distal cells contained cytoplasm and developed sterigmata and basidiospores (Fig. 6.5, E); (vi) typical basidia formed, but the sterigmata were thick, rounded and hyphalike and without basidiospores (Fig. 6.5, F). Basidia never formed more than two basidiospores.

Teliospore germination on spruce needles

As with glass slides, basidiospore deposition did not occur on spruce shoots when telia were suspended above young needles in a moist chamber. In freshly collected spruce samples, teliospores were abundant on the surface of immature current-year needles adjacent to year-old needles infected with *C. weirii*. Various stages of spore germination were seen by both LM and SEM. A mucilaginous material that appeared to hold the spores to the host surface could be seen by SEM (Fig. 6.6, A). Although teliospores were more often clumped together in artificially inoculated samples, the characteristics of teliospore germination on artificially inoculated spruce needles were similar to those on naturally infected material. Teliospore germination occurred more slowly on host tissue than in water.

Two main forms of teliospore germination were observed on host tissue. (i) Where many teliospores were clumped together on the host surface, basidiospore formation was rare and basidia often stood out from the host surface and divided to form two cells separated by a constriction (Figs. 6.6, A, B). As seen by LM, 20.5 h after inoculation, the cells of the basidia had become round and separated into two sporelike cells. Small rounded cells that may have resulted from fragmentation of basidia were sometimes also seen on the needle surface by SEM; they were smooth-walled, larger than basidiospores, and had a depressed center, perhaps resulting from shrinkage during preparation (Fig. 6.6, B, lower cell). (ii) Elongated basidia formed along the host surface and produced two sterigmata and basidiospores (Figs. 6.6, C, D). Basidiospores were often seen on the needle surface. These spores were globose with a tiny apiculus and a rough surface and were the same size as basidiospores produced on glass slides. Infrequently, basidiospores produced a germ tube or a secondary spore. Rarely, direct host penetration by a short germ tube was observed (Fig. 6.6, E). An appressorium was observed on only one germ tube. Some of the other types of teliospore germination seen on glass slides were also observed on host tissues (Fig. 6.6, F).

Cytology

The nuclear condition of hyphae in spruce needle tissue was difficult to interpret, because nuclei were often diffuse or greatly elongated. The DAPI stain sometimes showed two small nuclei side by side across the width of a hypha, but with the greater detail revealed by hematoxylin staining these were confirmed to be two parts of one dumbbell-shaped nucleus joined by a narrow isthmus. Interpretation was further complicated by the presence of many small scattered nuclear fragments in cells rather than distinct nuclei (Fig. 6.7, A). Of the cells in which distinct nuclei could be discerned, about 75% were monokaryotic (Fig. 6.7, A) and 25% dikaryotic. Below the base of the telium, globose pseudoparenchymatous cells were dikaryotic or monokaryotic. The thin-walled cells bounding the sorus at the sides contained one small nucleus, usually at the periphery. In the sorus, about 30-50% of the basal cells in the chains of teliospores were

dikaryotic; the rest were monokaryotic (Fig. 6.7, B). Teliospores of *C. weirii* contained one large diffuse nucleus that occupied up to one-quarter of the spore volume (Fig. 6.7, C). When a teliospore germinated (Figs. 6.7, D–I), the single nucleus migrated into the forming basidium (Fig. 6.7, D), where it divided near the proximal end. The nuclei then moved apart (Fig. 6.7, E) and a septum formed across the basidium (Fig. 6.7, F). As the sterigma and basidiospore began to form, the nucleus in each basidial cell divided again, and both nuclei moved, one at a time, into the basidiospore along with the cytoplasm (Figs. 6.7, G, H). Mature basidiospores most commonly contained four nuclei (Fig. 6.7, I), but up to six tiny nuclei or nuclear fragments were sometimes present.

Where the basidium fragmented into two rounded cells, these cells contained one or two nuclei, but after long incubation (3 d), many small nuclear fragments were present, as in mature basidiospores. Long hyphalike promycelia generally contained one large nucleus, or one nucleus in each of two cells, if septate. The nuclear condition during other variations of germination was not observed.

DISCUSSION

Spore dispersal

In *C. weirii* the teliospores function as diaspores. This is supported by their ready separation and dispersal in water, and their presence on field-collected current-year needles adjacent to year-old infected needles. Although the ready separation of the teliospores has previously been noted (Jackson 1917), the significance of this feature to the epidemiology of the fungus has not been recognized. I propose that dissemination of *C. weirii* occurs mainly through water dispersal of the teliospores rather than by wind dispersal of basidiospores produced in the sorus, as previously assumed (Weir 1923, Ziller 1974). A water-dispersal mechanism is consistent with both the teliospore morphology of this fungus and its distribution pattern. The teliospores are wettable, and they have a smooth surface, thin hyaline wall, and elongate shape, common features of splash-dispersed fungi (Fitt and McCartney 1986, Fitt et al. 1989). In contrast, wind-dispersed spores, such as the aeciospores of heteroecious *Chrysomyxa* species, are borne

dry, are non-wettable, have an ornamented surface, and are generally more rounded (Fitt and McCartney 1986). When dry, the spore masses of *C. weirii* form rigid crusts on the needle surface and in the sorus, but when water is present these masses dissolve, suggesting that an adhesive substance holds them together and prevents their removal by wind (Gregory et al. 1959). Such a substance would also protect the spores from desiccation during dry weather (Fitt et al. 1989). A few drops of moisture from mist, fog, or drizzle may be enough to float the spores from the sorus to the needle surface. Subsequent droplets from rain or dripping from branches above would further disperse the spores to susceptible young needles. *Chrysomyxa weirii* is mainly confined to the lower branches of mature trees, and individual trees become infected year after year while neighboring trees remain free of infection (Weir 1923). The sporadic distribution of this rust is consistent with the fact that dispersal by water occurs over much smaller distances than by wind (Fitt et al. 1989). During this study, I observed *C. weirii* in four locations in the Rocky Mountains of western Alberta. In all cases, the rust occurred on isolated trees or groups of trees in damp places near rivers or streams, where, in addition to rain, water-splash and heavy mist might provide enough moisture for dissemination and germination of the teliospores. Bergdahl and Smeltzer (1983) noted the correlation of infection with a period of rainfall in a blue spruce nursery. This dispersal mechanism should be an important consideration in the spacing of trees and in the method of irrigation chosen for spruce orchards where infection with *C. weirii* is prevalent.

This report of dispersal of rust teliospores by water appears to be unique. Dispersal of urediniospores by water, however, has been studied in several rust fungi (Rajasab and Rajendran 1983, Savary and Janeau 1986, Zadoks 1988).

Spore germination and nuclear cycle

It is apparent that *C. weirii* has a number of germination strategies, depending on the environmental conditions. Most teliospores produced basidiospores at 4 C, a temperature at which these delicate spores would be unlikely to be harmed by desiccation. At room temperature, however, teliospores were much more likely to form

fragmenting or hyphalike promycelia. Observations of naturally infected needles confirmed that these variations also occur under natural conditions. The slightly thicker walls of the basidial cells may render these propagules more resistant than basidiospores to the harmful effects of ultraviolet light or dessication.

It also appears that most basidiospore production does not occur in the telium. For seven other *Chrysomyxa* species from western Canada, teliospore germination and production of basidiospores has been readily obtained by enclosing infected plant material in a moist chamber in the same manner as described herein (P. E. Crane, unpubl). The absence of basidiospore deposition by this method with *C. weirii* and the ready germination of teliospores when dispersed in water suggests that basidiospore formation in *C. weirii* occurs primarily after teliospore dispersal and that free water is required for teliospore germination. Although basidiospores were occasionally seen within the mass of teliospores from a sorus, the globose or irregular spores most often observed did not have an apiculus and were slightly larger than basidiospores. They were identical with teliospores in pigmentation and morphology except for their size. They may have been unusually small teliospores or basidial cells resulting from fragmentation of the basidia.

The nuclear cycle in macrocyclic rust fungi is remarkably uniform; however, considerable variation in this pattern has been documented in microcyclic rusts (Jackson 1931, Petersen 1974, Hiratsuka and Sato 1982) . Most rust fungi produce four-celled basidia, but in this study of *C. weirii* the basidia were consistently two-celled except for a few cases where they were one-celled or four-celled, but in the latter case only two cells were functional. A photograph of *C. weirii* by Bergdahl and Smeltzer (1983) also appears to show a two-celled basidium. Weir (1923) claims to have observed four-celled basidia in *C. weirii* from which a single basidiospore was produced. It is possible that Weir misinterpreted the knoblike immature basidium as a basidiospore. The two-celled basidium has not previously been reported in the genus *Chrysomyxa*. However, a two-celled basidium may not in itself be unique within a genus (Oberwinkler 1982), because species with two-celled basidia are thought to be derived from simplification of forms

with typical four-celled basidia. For example, *Kunkelia nitens* (Schw.) Arth. on *Rubus* spp. lacks spermogonia and develops a two-celled basidium, but is likely derived from *Gymnoconia peckiana* (Howe) Trott., which has four-celled basidia and spermogonia (Dodge 1924, Cummins and Hiratsuka 1983). The two-celled basidium has been reported in several other microcyclic rusts, for example, *Uromyces aloes* (Cke.) Magn. (Thirumalachar 1946), *Frommea obtusa* (Strauss) Arth. (Cunningham 1966), and *Puccinia rutainsulara* D. E. Gardner (Gardner 1994). Some of the observed variations in *C. weirii* teliospore germination, such as the disarticulating basidial cells and hyphalike promycelia have also been reported in other rusts, either as aberrant forms under conditions of excessive moisture or low oxygen (Thirumalachar 1946, Petersen 1974, Cunningham 1966) or as the usual mode of germination (Pady 1935, Gardner 1988).

Based on the cytological study of *C. weirii*, the nuclear cycle is most likely as follows (Fig. 6.8). The nuclei in the vegetative hyphae are haploid; dikaryotization occurs at the base of the sorus, followed by nuclear fusion. The single nucleus in the teliospore is much larger than in other parts of the life cycle, suggesting that it is diploid and the product of karyogamy. During germination, the teliospore nucleus migrates into the basidium, where it divides. Two interpretations of the events in the basidium and basidiospores are possible, as follows. (i) The first division in the basidium is meiotic, and the second meiotic division is delayed until after septum formation in the basidium. The two products of the second division in each of the basidial cells both enter the forming basidiospore, where they subsequently divide mitotically to produce a tetranucleate basidiospore. Delay of the second meiotic division until after septum formation in the basidium has been reported in other rust fungi (Dodge 1924, Jackson 1935, Petersen 1974, Kohno et al. 1977). (ii) The first division in the basidium is mitotic, then a septum forms. The first meiotic division then occurs in each basidial cell, producing two nuclei per cell. These both migrate into the basidiospore, where they undergo the second meiotic division. In either case, tetranucleate basidiospores would result, the most common condition observed. Accounts of more than one nucleus in basidiospores are not unusual (e.g. Allen 1933, Berkson and Britton 1969, Petersen 1974,

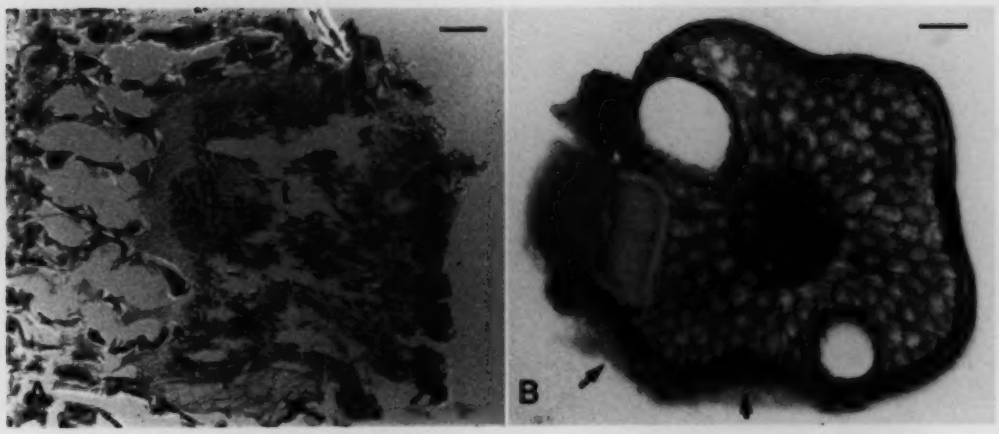
Kohno et al. 1977, Gardner 1987, 1988, 1994). The advantage to the fungus of having all products of meiosis in the same basidiospore is unknown, but also occurs in the microcyclic *Uromyces alyxiae* Arth. (Gardner 1987). Studies of the nuclear condition of germinating spores on the host surface (not observed in this study) would clarify the mechanism for transition from the tetranucleate basidiospores to the uninucleate mycelium in the host.

The presence of thin-walled bounding cells at the sides of the telia of *C. weirii* requires explanation, since a telial peridium is not characteristic of *Chrysomyxa* species. These thin-walled monokaryotic cells may not be a true peridium, but are likely remnants of a mass of fungal tissue in the host before telium initiation. They appear to be much like the undifferentiated cells that form a "protoaecium," as defined by Buller (1950) or like the "dermal layer" surrounding the telia of *Puccinia rutainsulara* (Gardner 1994). These cells contain a single small nucleus and were probably part of the monokaryotic mycelium present before initiation of teliospore formation by dikaryotization and karyogamy. They are unlike the modified spores produced in a catenulate manner that form a peridium around the aecia of macrocyclic *Chrysomyxa* species.

Relationship of C. weirii *to other members of* Chrysomyxa

The results of this study raise questions about the relationship of *C. weirii* to other members of the genus *Chrysomyxa*. In many rust genera, it is possible to correlate microcyclic species with a macrocyclic species of similar morphology, and from which the short-cycled form is presumed to have arisen (Jackson 1931). The morphology of *C. weirii* and the new information on its biology presented here do not suggest correlation with known heteroecious species of *Chrysomyxa*. Although separation of mature teliospores has been reported in the Asian species *C. deformans* (Diet.) Jackzew. (Dietel 1890) and in *C. roanensis* (Chapter 2), dispersal of their teliospores before germination has not been documented. Although the telia of *C. weirii* superficially resemble the telia of other species of *Chrysomyxa*, further studies are needed to determine whether placement in this genus is appropriate.

Fig. 6.1. Sections of spruce needles infected with *Chrysomyxa weirii.* (A) Telium (*t*) and large thin-walled fungal cells (arrows) adjacent to sorus (longitudinal section from paraffin-embedded material stained with hematoxylin). Bar = 50 μm. (B) Cross section of needle showing telium (*t*) and teliospore mass (arrows) crusted on needle surface. Bar = 100 μm.


B

Figs. 6.2–6.5. Spore morphology and germination of *C. weirii* on glass slides. **Fig. 6.2.** Chain of teliospores as formed in sorus, and some variations in spore shape. *b*, knob that will expand to become the basidium; *t*, tail-like extension of hyaline wall. **Fig. 6.3.** Most common mode of teliospore germination. (A) Basidium expands from knoblike process on end of teliospore by movement of cytoplasm from teliospore. (B) Septum forms in fully formed basidium. (C) A sterigma forms on each basidial cell. (D) Cytoplasm moves out of basidial cells into two expanding basidiospores. **Fig. 6.4.** Ungerminated basidiospores, and basidiospores germinating to produce secondary spore (*s*), short germ tube (*g*), or appressorium-like swelling (*a*). **Fig. 6.5.** Other types of teliospore germination, more common at room temperature than at 4 C. (A) Basidium fragments into two sporelike cells. (B) Hyphalike promycelium with two distal sporelike cells. (C) Germ tube on nonseptate promycelium. (D) Hyphalike promycelium with single sterigma and basidiospore. (E) Three-septate promycelium with the two distal cells producing basidiospores. (F) Septate basidium producing germ tubes. Bar = 10 μm.

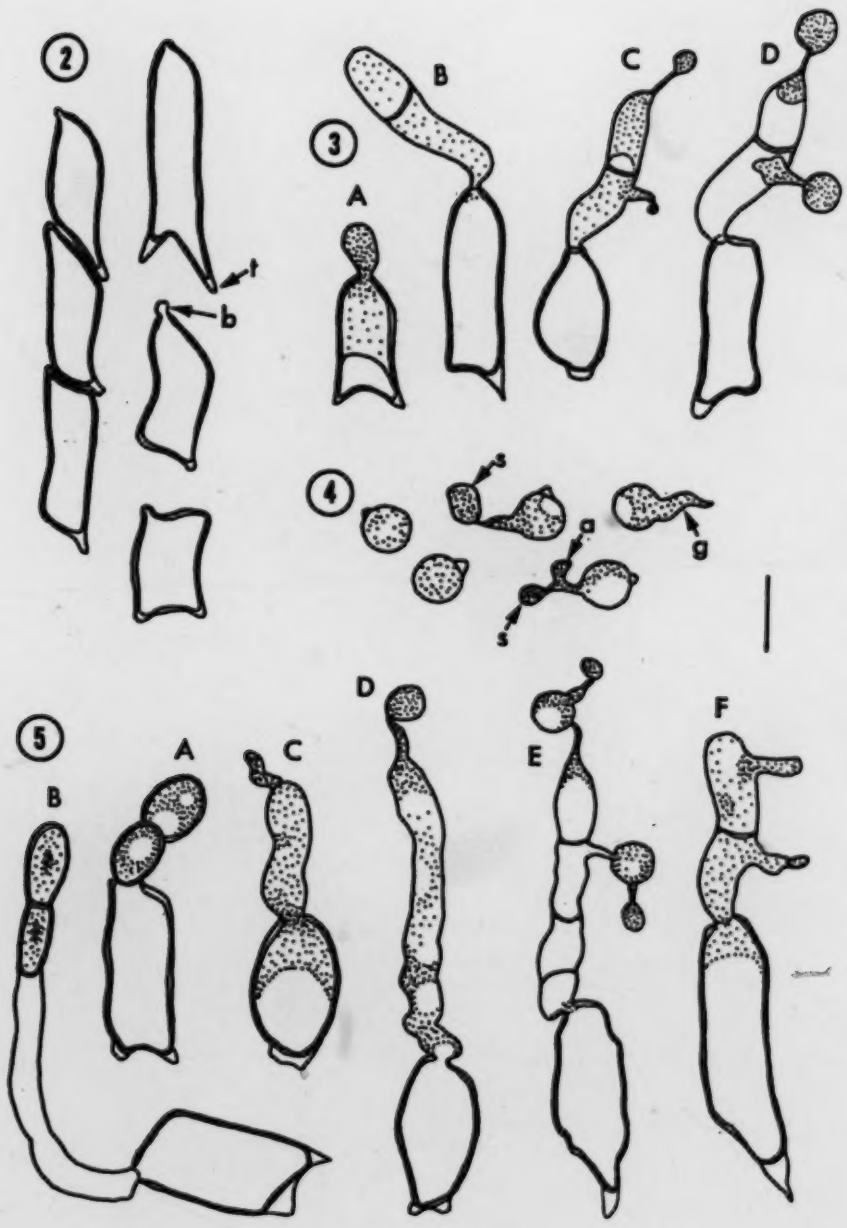

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Fig. 6.6. SEM micrographs of *C. weirii* on spruce needles of the current year. (A) Teliospores germinating to produce two basidial cells. Note mucilaginous material (arrow) at contact between spore and host. (B) Disarticulating basidial cells (arrows) at a later stage of development than in A. (C) Teliospores with elongate basidia (arrows). (D) Collapsed teliospore and basidium that has already released its basidiospores. Arrows indicate sterigmata. (E) Basidiospore with short germ tube that appears to be directly penetrating the needle surface. (F) Teliospores (*t*) with long hyphalike promycelia (short arrows). A–C are on artificially inoculated needles; D–F are on naturally infected needles.

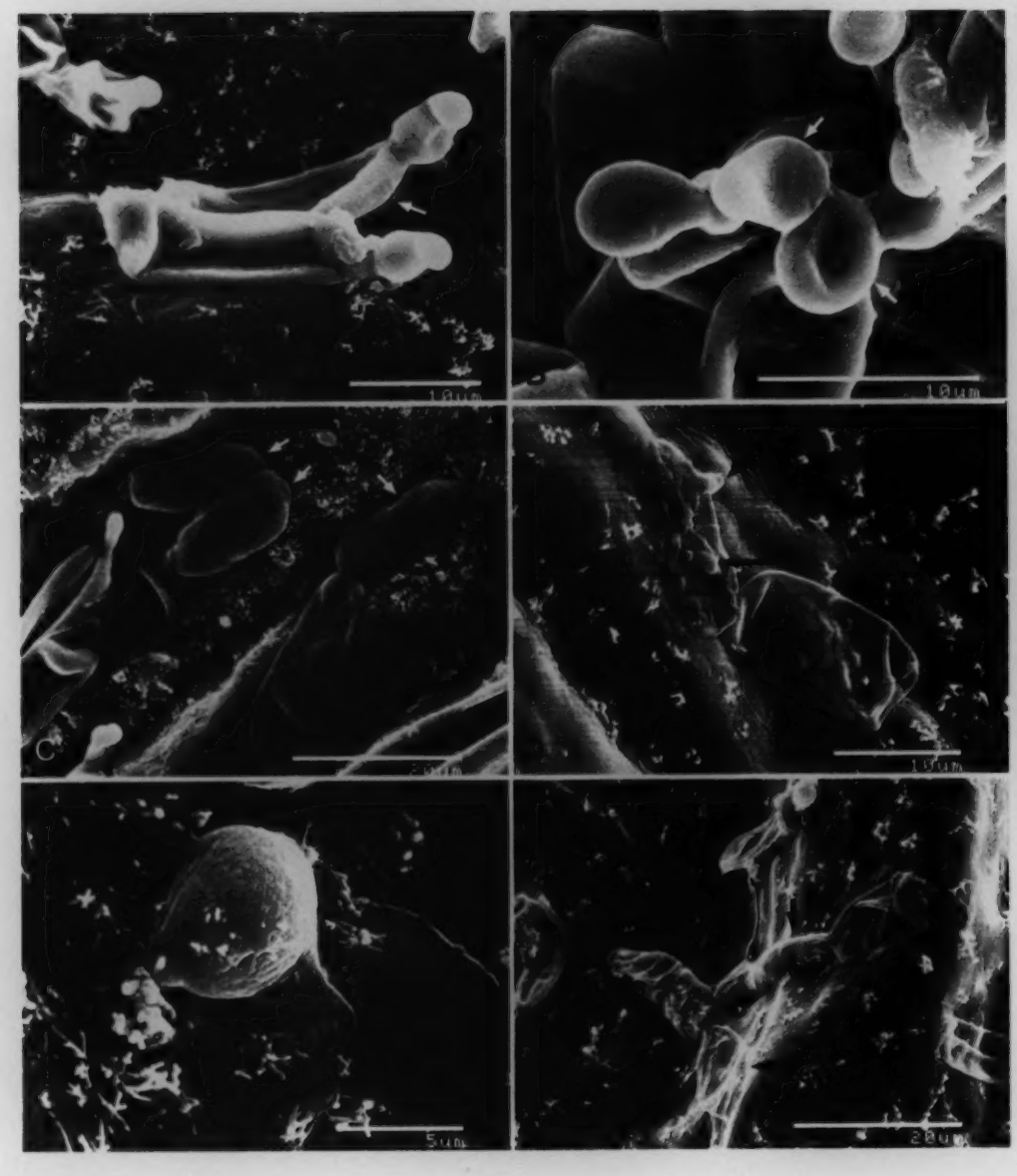
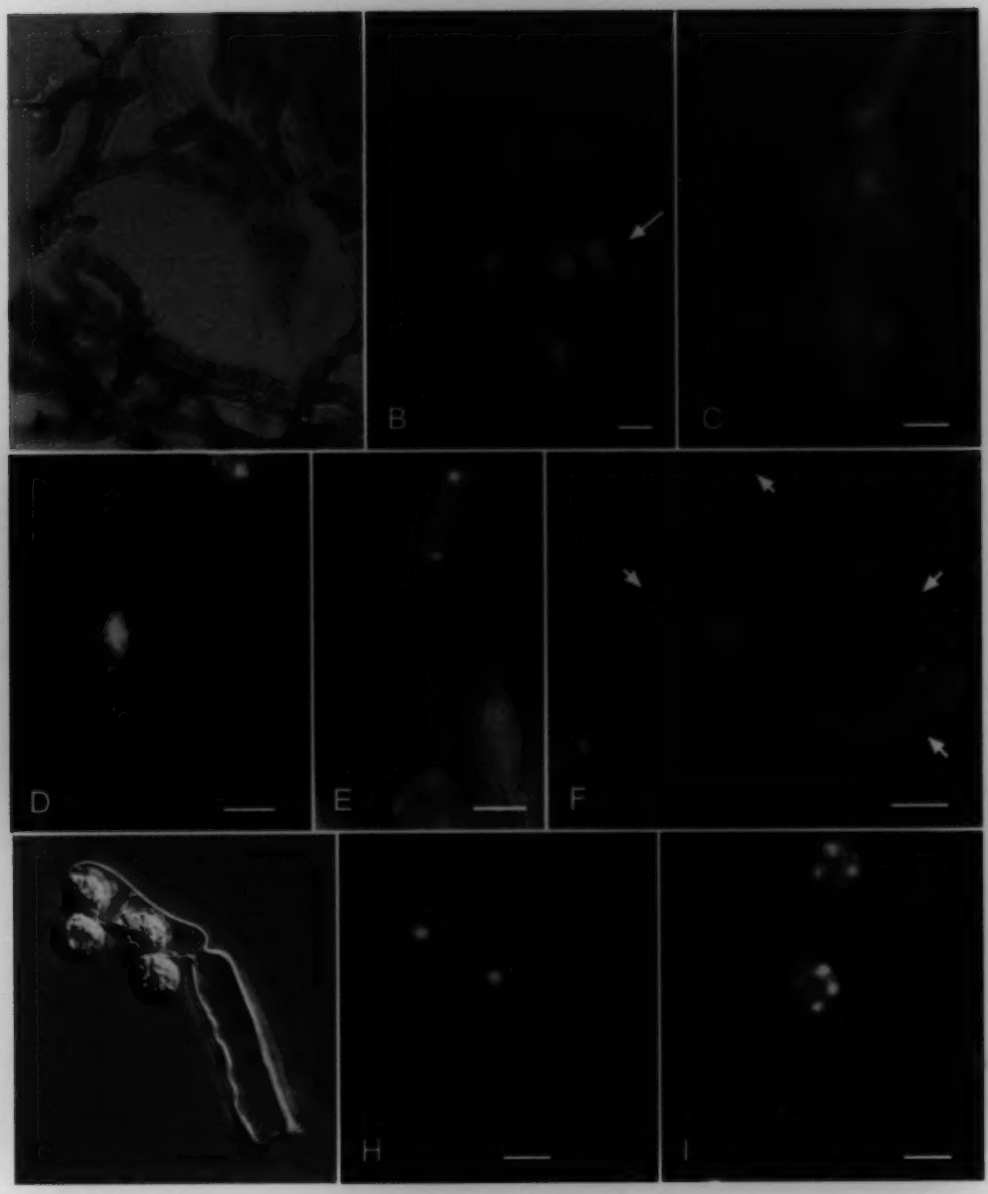


Fig. 6.7. Nuclear cycle of *C. weirii*. (A) Monokaryotic hyphae in cross section of infected spruce needle, stained with hematoxylin. Arrow indicates a hyphal cell with many small nuclear fragments. (B) Monokaryotic and dikaryotic (arrow) cells at base of teliospore chains. (C) Mass of monokaryotic teliospores. (D) Germinating teliospore. Nucleus has migrated from teliospore to basidium. (E) Nucleus has divided, and the two nuclei have moved apart. (F) Septa (arrows) have formed in several basidia. (G) Germinated teliospore and basidium, photographed using differential interference contrast optics. Cytoplasm is moving into the forming basidiospores. (H) Same as G, but under epifluorescent lighting to show DAPI-stained nuclei. (I) Fully formed basidiospores containing four nuclei (fourth nucleus in each spore is slightly out of focal plane). B–F, H, I, stained with DAPI. Bars: A, B, D–I = 5 μm; C = 7 μm.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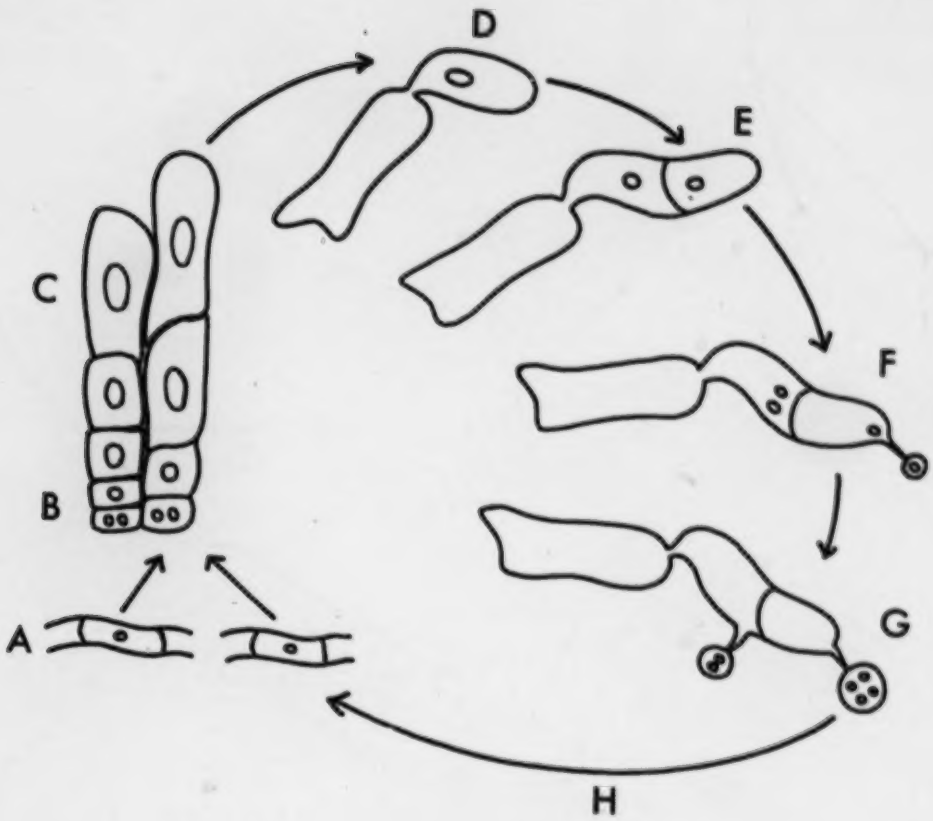

Fig. 6.8. Schematic drawing showing the major nuclear events in the life cycle of *C. weirii.* (A) Monokaryotic hyphae in spruce needle tissue. (B) Binucleate cells at base of sorus after dikaryotization. (C) Teliospores in sorus, each containing one large diploid nucleus after karyogamy. (D) Germinating teliospore; nucleus has migrated into basidium. (E) Diploid nucleus has divided, either by mitosis or the first meiotic division, and a septum has formed across the basidium. (F) Nuclei in basidial cells divide again and move into the forming basidiospores. (G) Nuclei in basidiospores divide again either by the second meiotic division, or by mitosis to form tetranucleate basidiospores. (H) Basidiospores germinate and infect new host needles. The mechanism by which multinucleate basidiospores result in uninucleate hyphae in the host was not observed.

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Chapter 7. Conclusions and future research directions

This study began with the goal of compiling a monograph of the genus *Chrysomyxa* worldwide, and with the assumption that host relationships and basic biology were fairly well-known for the species in North America and Europe. It has ended with the knowledge that important information is lacking about species delineations, intraspecies variation, and life cycles for even the most common of these obligate parasites. It is hoped that this study will contribute significantly to filling these information gaps and in suggesting new lines of study. Such knowledge is essential for an understanding of the relationship of these obligate parasites with their hosts and with other organisms, their modes of dispersal, the accurate identification of disease outbreaks on economically important crops, and in developing effective, rational disease control methods.

Systematic considerations

The use of scanning electron microscopy was crucial to the systematic study of the European and North American species of *Chrysomyxa*. This tool has elucidated the fine details of the surface ornamentation of the urediniospores and aeciospores, characters essential to defining species. Shape of warts, presence or absence of connections between them, and presence or absence of a longitudinal groove, cap, or flat area on spores were shown to be reliable features for distinguishing among species. In addition, the ornamentation of the aecial peridial cells was often helpful in this regard. The systematic study has confirmed the definition of many species and re-defined others. Particularly important is the clarification of the relationship of the European and North American species on *Ledum* and *Rhododendron*. The hypophyllous *Ledum* rusts in North America were shown to be distinct morphologically from the European *C. ledi*. With the increasing movement of host plants, both *Picea* and ericaceous hosts, to new locations, recognition of these distinct species is important to disease diagnosis and control.

The finding of a previously unknown, widespread rust, *C. reticulata*, on *Ledum groenlandicum* in North America may indicate that there has been an overreliance on host

plant specificity to identify rusts. Careful microscopic examination should precede an identification, especially for species of *Chrysomyxa* that infect *Rhododendron* and *Ledum*. *Chrysomyxa reticulata*, though apparently a minor component of the rust flora of native *Ledum* spp., is capable of causing economically important disease on imported cultivated rhododendrons. It is unfortunate that this species was not recognized earlier, as it may already have been spread to other areas of the world on rhododendrons (Bennell 1985). Serious outbreaks of rust disease may occur in situations where contact occurs between the pathogen and novel, but closely related, hosts.

The extreme variability in morphology and life cycle of *C. ledicola*, the most common spruce needle rust in North America, was documented for the first time. More study is needed to determine the nomenclatural implications of these variations.

In this study, it was possible to confirm, by artificial inoculation, the life cycles of *C. arctostaphyli*, *C. cassandrae*, *C. nagodhii*, *C. ledicola*, *C. reticulata*, and *C. woroninii*. However, for *C. ilicina*, *C. ledicola* (west coast variant), *C. roanensis*, *C. vaccinii*, and *Peridermium zilleri* such experiments have not been done, and are needed to complete the picture for the North American species. In addition, a thorough investigation of telial ontogeny and morphology of *C. ilicina* is needed to determine whether this species, with its atypical telial host (Aquifoliaceae), truly belongs in *Chrysomyxa*.

Value of basic biological studies and field observations

Field and experimental studies on the basic biology of several species of *Chrysomyxa* have presented new information that clarifies misconceptions about life cycles and mechanisms of dispersal. It is usually assumed that uredinial stages of rusts repeat during the growing season. This is not the case for most species of *Chrysomyxa* that were observed in boreal forests of western Canada. After old diseased leaves have been shed, new infections on current-year leaves exist within the host tissues as mycelium. There are few disease symptoms until the next spring, when telia and/or uredinia form. This "dormancy" may be caused by a resistance mechanism of the new leaves or it may reflect an adaptive mechanism by which the rust "allows" the host to

build up reserves during a short northern growing season, and thus ensures its own survival. Either way, it implies a long association of the hosts and pathogens.

Although many rust fungi have elaborate spore morphology, few studies have been done to correlate form with function, even for economically important pathogens. Novel mechanisms of spore dispersal have been suggested by the studies of *C. nagodhii* and *C. weirii*. The possibility that *C. nagodhii* is dispersed on its broadleaved hosts by Oribatid mites would explain the unusual urediniospore surface morphology (sticky and nearly smooth). Similarly, *C. weirii* was shown to have several unusual lifestyle adaptations: long, thin-walled teliospores that disperse and germinate readily when free water is present; a two-celled basidium, reflecting modifications to the nuclear cycle; and several modes of teliospore germination, probably adaptations to varying weather conditions. The evidence for water dispersal of the teliospores provides a new understanding of the sporadic distribution of this autoecious spruce rust, and suggests the possibility of effective control mechanisms for the disease. Neither the morphology nor cytology suggests correlation with other North American species of the genus, and further studies will be necessary to determine the affinities of *C. weirii*.

There is a need for a new understanding of the physiological and environmental factors that control sorus and spore production in rust fungi. Without this knowledge, many efforts to control economically important rust diseases may be inefficient and misdirected. The field and laboratory study of telial production in *C. pirolata* began with the simple observation that a greater proportion of telia were developing in *Pyrola* plants in a wet than in a dry area. Repeated field observations, and laboratory and field experiments confirmed the correlation of telial production with moisture. This phenomenon, which should be investigated in other locations where spruce cone rust is prevalent, may lead to a better understanding of year-to-year variability in disease levels.

Biogeography

The North American species of *Chrysomyxa* are interesting from a biogeographical point of view. This continent has obviously been a center of

diversification for the genus, with at least 10 unique species. Several, such as *C. weirii* have disjunct natural distributions, and likely survived in small eastern and western relict populations during glaciation or as a result of expanding grasslands. It is also possible that two separate autoecious forms arose independently. *Chrysomyxa roanensis* and *C. piperiana*, which have similar spore morphology and occur on native rhododendrons, but on opposite sides of the continent, likely arose fairly recently from a common ancestor that had a much wider distribution. The occurrence of an endemic species (*C. piperiana*) in California is not surprising, since this area has a high number of endemic plant species resulting from extreme differences in topography and climate within short distances (Lewis 1972). Similarly, the rusts of coastal British Columbia, including Vancouver Island and the Queen Charlotte Islands, probably diverged recently from more widespread ancestral species with which they are clearly connected: *C. vaccinii* from *C. chiogenis*, *C. monesis* from *C. pirolata*, and the west coast variant from more "typical" *C. ledicola*. The Queen Charlotte Islands are thought to have been a refugium during the Pleistocene glaciation, based on the large number of endemic plants and topography of the Islands (Calder and Taylor 1968). Isolation and lack of their accustomed host plants may have driven the morphological adaptations of these rusts: *Pyrola* is absent or rare on the Queen Charlotte Islands (Calder and Taylor 1968), and thus the coastal cone rust, *C. monesis*, has become specialized to only *Moneses* (Ziller 1954); *Gaultheria hispidula* is also lacking in the area (Calder and Taylor 1968), and therefore *C. vaccinii* has adapted to a related plant in subfamily Vaccinioideae, *Vaccinium parvifolium*. In addition, *P. sitchensis* is the only available spruce host in this part of the continent, leading to further host specialization (Farrar 1995).

Rusts as "plant taxonomists"

Specificity of rust fungi to their host plants has frequently been used to speculate on the relationships of host plants or to deduce the evolutionary history of the hosts (Savile 1969, 1979, 1990; Anikster and Wahl 1979). In addition to the above examples from coastal British Columbia, the present study supports other recent systematic studies

on plant relationships. Overall, the specificity of the genus *Chrysomyxa* to Ericaceae supports the modern concepts of Ericaceae to include Rhododendroideae, Empetraceae, Pyroloideae, and Vaccinioideae (Anderberg 1993; Judd and Kron 1993, Cullings 1994; Kron 1996, 1997). This study also supports the placement of *Ledum* in *Rhododendron* (Kron and Judd 1990; Harmaja 1991), because the same rusts, *C. ledicola*, *C. nagodhii*, *C. reticulata*, and possibly *C. woroninii*, infect both genera. On the other hand, other host and rust relationships are puzzling. *Chrysomyxa woroninii* on *Ledum* and *C. arctostaphyli* on *Arctostaphylos* share similar aeciospore morphology and the systemic growth habit, although the hosts are in different subfamilies of Ericaceae. Molecular systematic studies of these rusts will be important in determining whether they share a common ancestor or whether the morphological characters have arisen independently in response to a similar systemic habit in the hosts.

Future studies

The preliminary investigations into the use of DNA-based methods provide a basis for future studies. Sequencing of genes other than the ITS region of rDNA or completely different techniques will be necessary to solve some of the problems encountered in this study. When combined with the morphological studies of *Chrysomyxa*, molecular systematic studies have great potential to answer many questions about the genus: determining the phylogenetic placement of autoecious species; completing life cycle information where the connection of spore states on alternate hosts is unknown or unproven; providing independent characters to test phylogenetic hypotheses presented in this study; and, when combined with phylogenetic information of the host plants, enhancing our understanding of the evolutionary relationships of these obligate parasites and their hosts.

Detailed morphological studies of the species of *Chrysomyxa* endemic to Asia will be critical to a complete understanding of the genus. For many of these species, basic information is lacking on life cycles and biology. In addition to southern Asia, Japan appears to be another center of diversity of the genus *Chrysomyxa*, with several

unique species, morphological variations on more widespread species (*C. ledi*, *C. rhododendri*), and similarity to at least one western North American taxon (*C. ledicola*, west coast variant). A thorough study of the species unique to these islands may provide new information about the origin and biogeography of the rust genus. Comparison of Asian species with those defined in the current study is needed to determine species delineations and relationships. In particular, comparison of the Asian species having stalked telia (*C. himalensis*, *C. succinea*, *C. stilbae*, *C. tsugae-yunnanensis*, *C. qilianensis*) with those from elsewhere that completely lack this character or exhibit a much-reduced form of this character (*C. abietis*, *C. pirolata*) is needed to determine the significance of telial variation to the circumscription of the whole genus.

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APPENDIX: Glossary of terms used to describe rust fungi and their relationship with host plants

Abaxial: The side away from the axis.

Adaxial: The side toward the axis.

Aeciospore: A dikaryotic rust spore produced in an aecium, as a result of dikaryotization; in *Chrysomyxa*, always produced on the conifer host.

Aecium (-ia): A fruit body (sorus) containing aecisopores, produced after the spermogonia and before the uredinium in the life cycle of a rust.

Alternate host: One of two kinds of plants on which a rust fungus must develop to complete its life cycle.

Amphigenous: Growing on all leaf surfaces.

Anamorph: An asexual ("imperfect") stage in the life cycle of a fungus having several types of spores.

Annulate: Having a series of transverse, ringlike bands or ridges one on top of the other.

Annulus (-i): One of the ringlike layers on the warts of urediniospores or aeciospores.

Apiculus: A short projection at one end of a spore.

Appressorium: A swelling on a germ tube or hypha, especially for attachment to the host in an early stage of infection.

Attenuate: Gradually narrowed, becoming smaller and thinner.

Autoecious: A rust fungus that can complete its entire life cycle on the same host.

Basidiospore: A spore produced on the outside of a basidium following karyogamy and (usually) meiosis.

Basidium (-ia): A structure bearing on its surface a set number of basidiospores (typically four), usually following karyogamy and meiosis.

Bullate: Having bubble-like swellings.

Catenulate: Formed in chains or end-to-end series, as the aeciospores of rust fungi.

Chlorosis (adj. chlorotic): Yellowing of normally green plant tissue due to disease.

Clavate: Club-shaped.

Correlated species: In Uredinales, a species derived by reduction of life cycle from a parent heteroecious macrocyclic species, or the parent species itself.

Determinate: Having a fixed, definite limit, cf. indeterminate.

Diaspore: Any unit of dissemination, e.g. a spore or mycelial fragment.

Echinulate: Covered with small or finely pointed spines.

Ellipsoidal: Elliptical in shape.

Epiphyllous: Growing on the upper, adaxial surface of leaves or needles.

Erumpent: Breaking through, bursting out.

Falcate: Curved like the blade of a scythe or sickle.

***Fide*:** According to.

Fusiform: Spindle-shaped; tapering at both ends.

Germ tube: A germination hypha put forth by a spore.

Globose: Rounded, spherical.

Haustorium (-ia): An absorbing organ originating on a hypha of a parasite that penetrates the host cell wall and invaginates the host cell plasma membrane.

Heteroecious: A rust fungus requiring two host plants for completion of the life cycle.

Holotype: The single element (specimen) on which the name of a taxon is based.

Hyaline: Colorless.

Hymenium: A spore-bearing layer of a fruit body.

Hypophyllous: Growing on the lower, abaxial surface of leaves or needles..

Indeterminate: Without definite margin of edge.

Intercalary cells: Sterile cells occurring between catenulate spores.

Lanceolate: Lance-shaped, of much greater length than breadth, and tapering.

Lectotype: An element (specimen) selected, in a later work, from the original material, where no holotype was designated for a taxon.

Lenticular: Lens-shaped.

Localized infection: Restricted to a small part of a needle, leaf, or other plant part; cf. systemic infection.

Macrocyclic: Having a life cycle that typically exhibits all five stages (spermogonia, aecia, telia, uredinia, and basidia) of the rust life cycle.

Microcyclic: Having a life cycle with only the telial stage (and sometimes spermogonia).

Mycelium: The mass of hyphae that make up the body of a fungus.

Nomen nudum: A scientific name published without a proper description.

Obovate: Reversely ovoid, the apex broader than the base.

Ovoid: Egg-shaped.

Pathotype (pathovar): A subdivision of a rust species characterized by a pathogenic reaction to one or more hosts.

Peridermioid: Having an aecium as in the form genus *Peridermium*, i.e. with a well-developed peridium and aeciospores that are catenulate and verrucose; usually restricted to rust fungi on gymnosperms.

Peridium: The outer enveloping membrane of a fruit body, such as aecia or uredinia.

Primary infection: The initial infection of a host by a pathogen that has completed a dormant period.

Probasidium (-a): The portion of the basidium in which karyogamy takes place; usually the teliospore.

Promycelium: A germ tube issuing from the teliospore in which meiosis takes place and that bears basidiospores; the basidium.

Protoaecium: A mass of haploid cells, which after diploidization, becomes a fruiting structure.

Pseudoparenchyma: An aggregation of compactly interwoven fungal hyphae that somewhat resembles the parenchyma of higher plants.

Pulvinate: Cushion-shaped.

Punctate: Marked with very small spots or projections.

Pyriform: Pear-shaped.

Reticulate: Covered with netlike ridges.

Rugose: Wrinkled.

Rugulose: Delicately wrinkled.

Secondary infection: An infection initiated by spores produced in a primary infection.

***Sensu lato (s.l.)*:** In a broad sense.

Septate: Having cross-walls.

Septum: A cross-wall in a hypha.

Sorus (-i): A structure producing spores.

Spermatium (-ia): A male gamete produced in a spermogonium; spermatia do not cause infection.

Spermogonium (-ia): A fruit body (sorus) containing haploid spermatia; produced after the basidium and before the aecium in the life cycle of a rust.

Stellate: Having a starlike appearance.

Sterigma (-ta): A small hyphal branch or structure that supports a basidiospore.

Striate: Marked with delicate lines, grooves, or ridges.

Subglobose: Almost spherical.

Syntype: One of several elements (specimens) cited by an author when originally proposing a name, but where no holotype was selected.

Systemic infection: Of a pathogen, spreading internally throughout the plant body or an entire organ of the plant (e.g. a bud or cone).

Teleomorph: The "perfect" (sexual) state of a rust fungus, i.e. the state producing basidia; in rusts, the telial stage.

Teliospore: A spore that germinates to produce a basidium (promycelium); in *Chrysomyxa*, they are produced in chains, are thin-walled, and germinate without dormancy.

Telium (-ia): The sorus in which teliospores form.

Thallic sporogenesis: Spore formation in which a spore differentiates from a whole cell, rather than from part of a cell.

Truncate: Ending abruptly, as though with the end cut off.

Urediniospore: A dikaryotic, repeating spore of the rust fungi; in *Chrysomyxa*, always produced on the broadleaved host.

Uredinium (-ia): The sorus in which urediniospores form.

Verrucose: Covered with small rounded or truncate processes or warts, like the aeciospores of most rusts.

Witches' broom: An abnormal bushy growth on part of a plant characterized by short internodes and proliferations of twigs; generally a reaction to a pathogen.